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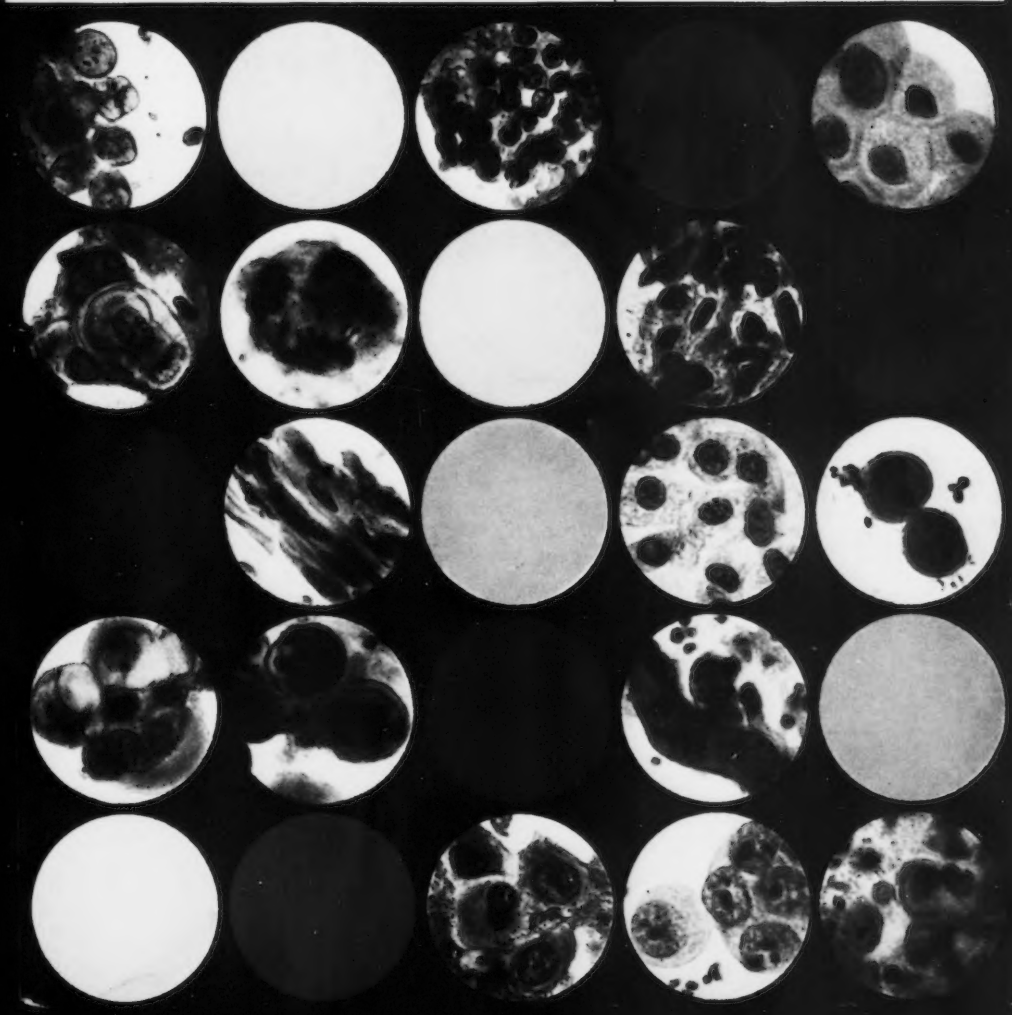
# Ca

## Bulletin of Cancer Progress



# *still unbounded potential*

This issue of *CA* is the second in a series of four offering carefully selected articles on exfoliative cytology by recognized authorities.

The American Cancer Society is remarkably privileged to have been closely associated for more than twelve years with the physicians and scientists who developed and fostered the broad use of exfoliative cytology in the diagnosis and treatment of cancer. No single development in cancer control in the half-century since the Society's founding has more readily arrested the attention and more promptly elicited the support of the Society. Enthusiasm for its promise and recognition of its results were early acquired from the teachings and writings of the Society's esteemed friend and modern cytology's principal benefactor, Dr. George N. Papanicolaou. Our firm conviction of the still unbounded potential of the method was strengthened by the enlightening work and dedicated devotion of the handful of physicians who recognized its merit at the outset.

The fact that during the term of cytology's development there were those who seriously questioned its value and took caution with its application has only enhanced its position today as a valuable aid to the early diagnosis of uterine and other cancers. Indeed, the effective and convincing demonstrations of cytology's usefulness and accuracy were sincerely stimulated by the less convinced and have now endowed the procedure with almost unchallenged acceptance. By the same process there has been careful refinement of the standards of its application and interpretation.

To all those who participated in the development of exfoliative cytology, to the "enthusiastic proponent" and the "demanding doubter," the American Cancer Society extends deep and continuing appreciation for their contributions.

Director of Service

*Cover—*

*Design by Don Smith, New York City.*
*Photomicrographs from the collection of Dr. G. N. Papanicolaou.*

# NEWSLETTER

JULY-AUGUST, 1960

At the recent fourth Canadian Cancer Research Conference in Ontario, sponsored by the National Cancer Institute of Canada and the Canadian Cancer Society, a number of reports on the viral origin of cancer were made. Dr. Ernest A. McCulloch (Ontario Cancer Institute) reported that the polyoma virus could produce many kinds of tumors in newborn mice and hamsters. Dr. Arthur Ham, an associate, reported that studies reveal that viruses can cause tumors without actually being found within the tumors. If it is eventually proved that human cancer is caused by viruses, it is hoped that a vaccine can be developed which will induce antibody formation.

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The University of Nebraska has been awarded a grant of $2,500,000 for the establishment of the Eugene C. Eppley Institute for Research in Cancer and Allied Diseases. The Institute will study the causes of cancer and allied diseases and seek to improve methods for diagnosis and treatment. A new six-story building to house the Institute wil be constructed in the fall.

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In a recent issue of California Medicine, a progress report of the Cancer Advisory Council of the State of California was made. This council was appointed by the Governor and its program, authorized by the State Legislature in 1959, is to protect the public against useless and harmful methods of cancer treatment.

Dr. John W. Cline, chairman, and his group met in April to determine the ways and means of collecting and testing alleged cancer cure methods. Now that the groundwork has been laid, it will be the committee's province to determine the value of irregular methods of cancer treatment. If a remedy is found to be worthless, the council has the authority to protect victims and their families from hardship and financial loss which might result from such quackery.

The growing need for nurses better prepared in cancer nursing, as well as for cancer nursing specialists, has led to the development of a program in cancer nursing education at New York University. This comprehensive program offers three types of study: a short-term course in oncologic nursing which accepts 20 students each session; a 12-month internship available to 40 students a year; and a course leading to a master's degree or doctorate in cancer nursing. Institutions which are cooperating in this teaching program include the Memorial Sloan-Kettering Cancer Center and the James Ewing Hospital of the City of New York Department of Hospitals. This project should provide educational opportunities to nurses at a variety of levels.

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At the recent UNESCO symposium on cell biology in Belgium, Dr. Walter G. Verly of the Department of Biochemistry, University of Liège, recommended that tritium be used to destroy cancer cells. He stated that tritium could be employed to label any compound containing firmly bound hydrogen atoms, including normal metabolites of the cell which are preferentially taken up by cancer cells. Its low energy of beta radiation and its mean penetration of only 1 micron in soft tissues make tritium of value in radiotherapy. Dr. Verly reported that tritium disintegrations irradiate cells in which the tritium is contained without producing much effect on adjacent cells.

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At the 14th annual Rocky Mountain Cancer Conference in Denver in July, sponsored by the American Cancer Society and the Colorado State Medical Society, Dr. Warren H. Cole, president of the American Cancer Society, predicted that within 10 or 15 years a chemical cure or immunologic vaccine for some types of cancer would be found. He stated, however, that it will probably be several decades before such cures will be known for all types of cancer.

At the same meeting, Dr. E. Vincent Askey, president of the American Medical Association, made a plea for earlier detection and treatment of cancer and proposed that industry reveal its statistics on occupational cancer so that our knowledge of cancer and its origin can be widened.

(Continued after page 144)

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# at a glance . . .

**one-minute abstracts of the literature on genitourinary cancer**

### Unsuspected Prostate Cancer

Microsections from 847 consecutive patients who had prostatectomies done at Barnes Hospital (St. Louis), from 1940 through 1953, were reviewed by the authors who had no previous knowledge of the clinical findings or original pathological diagnoses. Sixty-three cases of adenocarcinoma of the prostate were found and their clinical records were studied. In eight cases there had been clinical suspicion of cancer preoperatively. In the remaining 55 cases (6.5 per cent), with which this study deals, there was no suspicion of cancer and the preoperative clinical diagnosis was benign prostatic hyperplasia.

Follow-up information was obtained on all 55 patients with unsuspected adenocarcinoma of the prostate. The mean age of these patients was 71.3 years (for the 847 patients undergoing suprapubic prostatectomy it was 67.8 years). The crude five-year survival rate for the 55 patients is 54 per cent; in 24 of these who have been followed for 10 years, the survival rate is 37 per cent. It was found that the microscopic differentiation and the size of the cancer are directly related to survival. Patients who have small, well differentiated carcinomas have done well clinically and may not require further treatment. Their five- and 10-year survival rates are 75 per cent and 47 per cent, respectively. There is no evidence that antiandrogenic therapy has increased survival. In patients who have less than well differentiated carcinomas, the outlook is poor and there is no demonstrable benefit from orchiectomy or estrogens. Their five- and 10-year survival rates are only 33 per cent and 14 per cent, respectively. The authors conclude that patients with well differentiated tumors found on suprapubic prostatectomy need not have secondary radical prostatectomy. For patients with moderately or poorly differentiated cancers discovered on suprapubic prostatectomy, however, secondary radical prostatectomy may be a rational therapeutic approach.

*Bauer, W. C.; McGavran, W. H., and Carlin, M. R.: Unsuspected carcinoma of the prostate in suprapubic prostatectomy specimens; a clinicopathological study of 55 consecutive cases. Cancer 13:370-378, Mar.-Apr., 1960.*

### Renal Cancer and Polycythemia

The presence of renal cell tumor or hypernephroma is shown by clinical symptoms which seem in no way related to the growth. The awareness that renal tumor produces symptoms other than the old classical triad of hematuria, growth and pain should reduce the time interval between diagnosis and surgery. This paper presents three patients with renal tumor in

whom polycythemia occurred. The author reports that the association of polycythemia-like states with renal tumors is another manifestation of this disease. The polycythemia theoretically may be due to erythropoiesis occurring in the tumor or surrounding renal tissue, may be secondary to arterial oxygen saturation, or may be caused by erythropoietic substance produced in the tumor. It is recommended that all patients with polycythemia-like states should have a urologic survey consisting initially of excretion urograms and repeated cell studies of urines.

*Ewert, E. E.: Renal cell carcinoma and polycythemia. S. Clin. North America 40:767-770, June, 1960.*

### Estrogen-treated Prostatic Cancer

This paper describes the changes found at autopsy in 52 cases of clinical prostatic cancer in patients who were treated with natural or synthetic estrogens for periods of two weeks to nine years. The age distribution, response to treatment, cause of death, and survival period of the 52 patients were studied. The age incidence and survival period after treatment are similar to those found in other series. Nineteen patients died from intercurrent disease; 12, as a result of metastases; 13, from urinary obstruction or infection; and eight, of postoperative complications.

The cases were divided into four groups histologically. In the first group of six patients, the tumors showed no degenerative changes after estrogen treatment; the histologic structure was identical with that of untreated tumors. In the second group of 10 patients, the cancer cells in the primary tumor and secondary deposits were largely inactive and the structure of the tumors was distinctive. In the tumor cells, intracytoplasmic vacuoles coalesced causing a swollen "balloon" appearance with the nuclei pushed to one side; these cells were desquamated and filled the alveolar lumina. The nucleus became condensed, hyperchromatic and then pyknotic. Later the swollen cells ruptured. The glandular stroma became vacuolated with fibrillar basophil strands. Many tumor cells were completely replaced by fibrosis, but some remained in small clumps. In the third group of 10 patients, the primary tumor was apparently inactive, but metastatic deposits were rapidly growing. In the fourth group of 26 patients, active cancer cells were present in both the primary and secondary tumors, but there was evidence of previous estrogen-induced retardation of the tumor.

Not all prostatic tumors respond to endocrine treatment. The response to such treatment varies from tumor to tumor and from part to part of the same tumor. Even in seemingly inactive tumors, a few unaffected tumor cells can be found. It is apparent that hormone-induced retardation of tumor differs morphologically from that found in naturally occurring latent tumors.

*Franks, L. M.: Estrogen-treated prostatic cancer; the variation in responsiveness of tumor cells. Cancer 13:490-501, May-June, 1960.*

### Familial Aspects of Prostate Cancer

This study was done to determine whether a familial aggregation could be demonstrated for carcinoma of the prostate among a specific population in Utah, using as basis for comparison the incidence in the general population of the state. Propositi were selected from death certificates, for the period 1942 to 1958, of white persons dying of prostatic cancer. Parents and sibs of the propositi were investigated through records of vital statistics of the Utah State Department of Health. The control group consisted of white persons of the same sex who had died in the same year, same county and at the same age as each of the deceased relatives of the propositi.

At completion of the study, 228 families had been analyzed. The ages of the propositi at their death ranged from 42 to 95; the average age was 74. For these families, 355 death certificates of the fathers and brothers were reviewed. Fifteen of these certificates gave prostatic cancer as the major or contributory cause of death; in the controls the number was five. Death certificates were observed for 304 of the female relatives of the propositi. Forty-three of their deaths were attributable to

cancer as compared with 41 among the controls.

The results are compatible with the hypothesis that prostatic cancer has a genetic component that is organ-specific. Deaths due to cancer were three times more frequent in the male relatives of the propositi than in controls. The number of other types of cancer deaths in the male and female relatives was not significantly increased over that observed in controls.

*Woolf, C. A.: An investigation of the familial aspects of carcinoma of the prostate. Cancer 13:739-744, July-August, 1960.*

## Cytohistologic Observations on Bladder Cancer

Cytologic examination of urinary sediment is a sensitive index of epithelial alterations in the renal pelvis, ureters and bladder and has led to the discovery of unsuspected carcinoma *in situ* of the urinary bladder in several patients at the Memorial Center for Cancer and Allied Diseases (New York). The technique is useful as a screening procedure for industrial workers exposed to bladder carcinogens.

Urinary sediment of voided urine from 285 men exposed to *p*-aminodiphenyl was examined. Three smears of centrifuged sediment from each specimen were prepared and stained by the Papanicolaou technique. In 31 men the sediment contained significantly abnormal epithelial cells indistinguishable from typical cancer cells; of these, 22 had a sufficient number of these cells to warrant a cytologic diagnosis of cancer; the remaining 11 were classified as "suspicious".

Histologic confirmation of carcinoma has been obtained in 12 of the 31 men with suspicious or positive cytological findings. In three instances (two of *in situ* and one of superficially invasive carcinoma) cystoscopic examination showed only patchy mucosal reddening. In one other case, biopsy of such an area revealed a borderline lesion, but this patient developed papillary carcinoma four months later. Similar cystoscopic abnormalities were found in seven other men but biopsies were not done. Eight had no evidence of tumor on at least one cystoscopy. The remaining 19 men who had cancer cells in their sediment are still without clinical evidence of cancer. In these cases the cells are indistinguishable from those confirmed as cancer cells by biopsy. It appears that latent periods of varying length occur prior to development of clinical bladder cancer. During such periods, cancer manifests itself at the cellular level and is detectable by the cytologic technique but may escape detection by cystoscopy and biopsy for months or years. These findings suggest that this latent period is comparable with that associated with *in situ* carcinoma of other organs.

*Melamed, M. R.; Koss, L. G.; Ricci, A., and Whitmore, W. F.: Cytohistological observations on developing carcinoma of the urinary bladder in man. Cancer 13:67-74, Jan.-Feb., 1960.*

## 5-Fluorouracil Therapy of Bladder Cancer

This paper is a preliminary report on the use of 5-fluorouracil (5-FU), a new antimetabolite, in the treatment of bladder carcinoma. The compound is a fluorinated pyrimidine and pyrimidine antagonist which specifically blocks the formation of DNA with resultant metabolic inhibition. It was chosen over other related compounds on the basis of experimental studies which indicated its potent antineoplastic activity *in vitro* and its low toxicity *in vivo*.

Ten patients with far advanced primary bladder carcinoma received a full course of 5-FU. Four of the 10 patients have expired, but all showed marked clinical evidence of tumor regression and in one this clinical impression was confirmed by tissue evaluation at autopsy. The remaining six patients have shown a good clinical response to initial therapy and several of them, who required repeated courses, have not shown any indication of development of drug fastness or cumulative toxicity. The average interval between courses of treatment has been 10 to 12 weeks.

Although the investigational period was short (10 months), the authors feel that their repeated observations of regression of tumor and its associated symptomatol-

ogy by treatment with 5-FU, and the relatively low toxicity and apparent lack of drug fastness make 5-FU an extremely valuable adjunct in the palliative therapy of advanced bladder carcinoma.

*Deren, T. L., and Wilson, W. L.: Use of 5-fluorouracil in treatment of bladder carcinomas. J. Urol. 83: 390-393, April, 1960.*

## Early Diagnosis of Male Genitourinary Cancer

Early diagnosis still offers the greatest immediate hope in the battle against cancer and it is the general practitioner who has the first opportunity to wield this weapon. Every physician should be equipped to make the correct diagnosis if a malignant condition does exist. The requisites for this are an awareness of the possibility of cancer, a familiarity with the clinical signs of the neoplasm in question, willingness to do careful history-taking and physicial examination and knowledge of the laboratory and roentgenologic aids which are available. How do these requirements find application in diagnosing neoplasms of the male genitourinary tract?

Cancer of the kidney has usually attained large size before clinical signs appear. The majority of renal neoplasms are adenocarcinomas, renal pelvic growths or Wilms' tumors, the first two occurring commonly after forty years of age and showing a predilection for the male sex. Wilms' tumors have greatest incidence in children between two and five years of age. Gross bleeding and lumbar pain are common in adenocarcinomas and renal pelvic growths but unusual in Wilms' tumors. The complaint of a mass is uncommon in adenocarcinomas or renal pelvic cancers but is present in about three quarters of children with Wilms' tumors. A palpable mass in the lumbar region helps to confirm suspicion but its absence does not rule out the possibility of renal growth. Most patients with renal cancers have abnormal findings on urinalysis, proteinuria being more frequent than microscopic hematuria. The Papanicolaou method for examining urinary sediment is a helpful diagnostic adjunct. The bulk of objective evidence for renal neoplasm, however, is usually derived from radiographic studies.

Bladder cancer often gives rise to symptoms early in its course. Most of these cancers occur in the fifth decade and commonly in males. Gross hematuria is the first evidence of the disease in three fourths of the cases and is usually accompanied by symptoms of bladder irritation. Physical examination may be unrevealing, but careful palpation of the bladder base through the rectum or vagina (with bladder empty) may reveal a mass or abnormal induration. Abnormal urinalysis findings of hematuria and pyuria are the rule; cytologic examination of the urinary sediment is also highly useful. Cystoscopy with biopsy, however, is the keystone of diagnosis of bladder cancer.

Cancer of the prostate is a common neoplasm which increases in frequency beyond the age of 50. Early prostatic cancer produces no symptoms. Any area of induration found on digital rectal examination should be suspect, and biopsy is indicated if the induration is not adequately accounted for by infection or calculus. Routine and periodic digital rectal examination of all men past the age of 50 continues to be the most practical and productive technique for detection of early prostatic cancer.

Cancer of the penis should be easily recognized. It occurs commonly in uncircumcised men past 40 with an antecedent history of phimosis with or without infection. Biopsy should be done of any papule, ulceration or keratotic plaque which does not heal promptly with therapy.

Testicular cancer is common in the age group between 20 and 40. Earliest symptoms are often painless testicular enlargement and dragging discomfort in the groin. An overlying hydrocele many conceal a tumor but aspiration of the hydrocele should permit accurate palpation of the testis. Transillumination is an aid in diagnosis. The differential diagnosis of scrotal masses is seldom difficult, but prompt surgical exploration is indicated in doubtful cases.

*Whitmore, W. E.: Early diagnosis of cancer of the male genitourinary organs. New York J. Med. 59:2228-2231, June 1, 1960.*

# Management of the Patient with the Positive Vaginal Cell Examination

*Harry M. Nelson, M.D., Esther H. Dale, M.D. and Gerald S. Wilson, M.D.*

In spite of the fact that cervical cancer is almost 100 per cent curable if found in an early stage, 15,000 women die of the disease each year. This is especially regrettable because of the easy, effective and accurate test that is available in the form of the "Pap smear" for detecting cervical cancer in an extremely early stage. In order to stimulate wider use of the test, the American Cancer Society is sponsoring a nation-wide cytology program which is designed to encourage women to have this test performed by a private physician in his own office.

One of the cancer detection centers in the United States which is contributing valuable statistics to this cytology program today is the Yates Memorial Clinic, Detroit, Michigan, established by the Southeastern Michigan Division of the American Cancer Society.

The Yates Clinic, which opened in May 1950, is staffed by members of the Wayne State University College of Medicine. Besides being of service to the people of the community, the primary function of the Yates Clinic is to carry out statistical research in the evaluation of various cancer detection tests.

Patients accepted for examination at the Yates Clinic are presumed to be asymptomatic and all are required to provide the name of a private physician to whom the results of the examination are sent. The patient is not advised as to the diagnosis or treatment; rather, she is requested to return to her private physician. Three months after her examination the Clinic sends a follow-up letter to her physician requesting information on any further diagnostic procedures and the ultimate management of the patient. When a patient is found to have proven or suspected cancer, the doctor is contacted directly without delay.

*From the Yates Memorial Clinic and Wayne State University, Detroit, Michigan.*

During a four and one-half year period beginning in May of 1950, 15,832 women had routine vaginal cell examinations at the Yates Clinic[1]; 160 had positive reports (Table 1). Biopsies were obtained in 140 of these women with positive cell examinations and 123 proved to have carcinoma by histological examination, an accuracy of 87.8 per cent. It deserves to be pointed out here that positive smears should always be confirmed by biopsy before therapy is initiated. Some cytologists, however, are of the opinion that the results of cell examination may prove to be more accurate than biopsy results because the material constituting the smear may be more representative and may contain neoplastic cells from an area missed by biopsy. A cold-knife conization, of course, diminishes the possibility of error in this regard.

The value of cytology as a screening procedure is further demonstrated by the number of diagnoses of cancer which are first suspected through vaginal cell examination when no clinical evidence of cervical malignancy is present. In a series of 147 cases of squamous cell carcinoma of the cervix diagnosed at the Clinic, 62 (42 per cent) were first detected by cell examination after being unsuspected by clinical examination (Table 2). If it were not for the cell examination, these cancers would have gone unrecognized and the most favorable time for therapy missed. Contrary to what might be assumed, not all of these cancers were preinvasive. Naturally, a high percentage (74.3 per cent) of the preinvasive cancers were first suspected by smear. It is significant, however, that even in those cancers which were histologically proved to be invasive, 23.1 per cent were first suspected by smear and the re-

Table 1

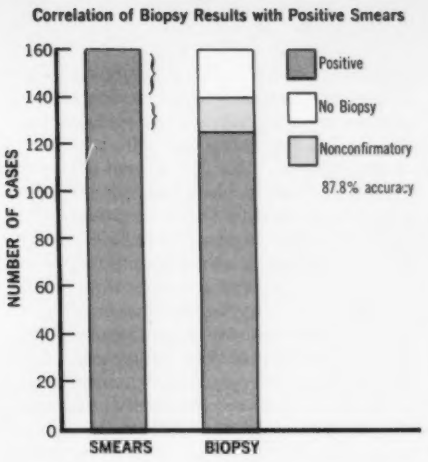
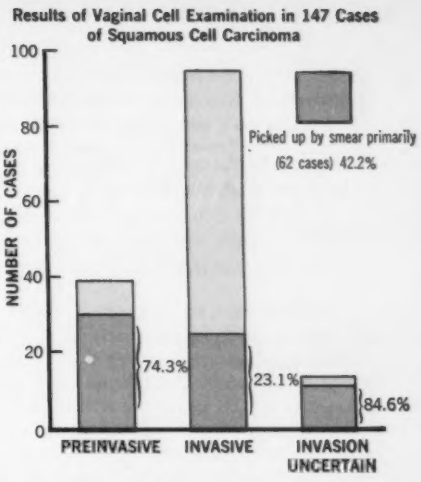



Table 2




maining 76.9 per cent were obviously malignant on clinical examination.

In an effort to establish more fully the value of the cytologic test and to study the causes of the so-called false positive smears, 100 consecutive patients with positive smears diagnosed at the Yates Clinic were followed from the initial examination to the ultimate diagnosis.[2] This was accomplished by first contacting the patient's doctor and encouraging him to perform additional biopsies, as well as dilatations and curettages, when indicated. In instances where the patient had undergone surgery, the pathological material was obtained and the microscopic slides were reviewed by the pathologists at the Yates Clinic. In addition to this extensive examination, smears and the biopsy material obtained at the Clinic were also studied carefully by a special panel of reviewing pathologists.

Of these 100 cases, 58 were confirmed immediately at the Clinic by simple punch biopsy but 42 were not confirmed; in 35 of the 42 that were unconfirmed, biopsies were negative and in seven no biopsies were performed (Table 3). During further study of these 42 cases, two of the patients who did not have biopsy performed at the Yates Clinic had punch biopsies done on follow-up examinations and were proved to have carcinoma. In 15 cases additional material from the cervix confirmed the cytologic diagnosis. This was accomplished in seven instances by cold-knife conization of the cervix, in one by additional punch biopsy and in seven by total hysterectomy and examination of the excised cervices by the pathologist. In three additional cases the carcinoma actually involved the endometrium rather than the cervix and was discovered only after a diagnostic curettage. In one patient the carcinoma involved the vulva and the resulting positive smear was from this lesion rather than from a uterine carcinoma. In six cases the biopsy material was examined by a group of pathologists and the diagnoses changed from benign to malignant disease. Twenty-seven of the 42 cases were thereby confirmed by biopsy and other follow-up procedures, leaving 15 still unaccounted for (Table 4). In eight of

Table 3

100 Consecutive Positive Pap Smears of Cervix

| | | |
|---|---|---|
| Pap smear confirmed by biopsy at Yates | | 58 |
| Pap smear not confirmed at Yates | | 42 |
| Biopsy negative | 35 | |
| Biopsy not done | 7 | |
| Total | | 100 |

Table 4

REASON FOR CHANGING NONCONFIRMED STATUS TO CONFIRMED POSITIVE

| | | |
|---|---|---|
| Biopsy not performed at Yates, but cervical biopsy positive on follow-up | | 2 |
| Additional biopsy material from cervix | | 15 |
| Cold-knife conization | 7 | |
| Punch | 1 | |
| Hysterectomy | 7 | |
| Carcinoma not in cervix | | 4 |
| Endometrium | 3 | |
| Vulva | 1 | |
| Review of biopsy by panel | | 6 |
| TOTAL | | 27 |

the remaining 15, review by a group of cytologists and pathologists resulted in downgrading the smear from a positive to "atypical" or "suspicious." In two instances no biopsy of the cervix was performed at the Yates Clinic or elsewhere, and in five cases adequate biopsies were obtained and properly interpreted as benign (Table 5).

The most common reason for initially failing to confirm the positive smear in this series was failure to obtain adequate biopsy. Of the original 42 discrepancies, 21 fell within this category. Positive smears may be confirmed by a simple punch biopsy done in a doctor's office. The biopsy specimen must be of adequate size. It must come from the involved area and should be handled carefully so as to be intact for pathological examination. Tissue which has been crushed or in any way damaged so that fine histological detail is no longer discernible must be considered inadequate. Any burning of tissue will also render the specimen unsuitable for histological diagnosis. Sometimes biopsy specimens are too small or are taken from a region too far out on the portio vaginalis when the involved area is near the internal os.

Table 5

NONCONFIRMED CYTOLOGIC DIAGNOSES

| | |
|---|---|
| Smears downgraded to atypical or suspicious on review | 8 |
| No biopsy | 2 |
| Adequate biopsies properly interpreted as benign | 5 |
| TOTAL | 15 |

When a patient has a positive smear it can be assumed that she has a malignancy and the burden of proof must be accepted by the clinician. If simple punch biopsy is inconclusive or negative, a four-quadrant punch biopsy may be the answer. If the positive smear is still unconfirmed, hospitalization for cold cone biopsy and dilatation and curettage must be considered. Three of the above-mentioned patients with a false positive smear proved ultimately to harbor an endometrial carcinoma.

Some physicians commonly make the mistake of dilating the cervix and curetting the endometrium before doing a cervical biopsy. Under such circumstances the biopsy is often inadequate because the surface epithelium has been removed from its normal position.

It should be emphasized that of the seven cases in which cold-knife conization of the cervix revealed a malignancy when the punch biopsy had appeared to be nonmalignant, four proved to be invasive carcinoma. This is a very important consideration for it is not generally appreciated that an invasive carcinoma of the cervix can be missed by punch biopsy. Of the seven cases in which the findings on hysterectomy changed the diagnosis, one turned out to be an invasive carcinoma of the cervix. In four cases the biopsy was inadequate because the wrong organ or tissue was biopsied; three of these were found to be carcinoma of the endometrium and the original biopsy specimens were from the cervix; the fourth case was a carcinoma of the vulva.

In eight of the 100 cases a review of the smears indicated that cell alterations caused by degeneration, improper fixation or infection had been mistakenly diagnosed as malignant change. It is important, therefore, that cytologists be well experienced, since a good understanding of the cellular changes associated with various benign reactions will decidedly reduce the

number of true false-positive diagnoses.

Another facet of this problem which deserves emphasis is the matter of evaluation of tissue changes present in the biopsy. It is regrettable, but nevertheless true, that the diagnosis of carcinoma *in situ* is to some extent still a subjective matter. The definition of noninvasive carcinoma utilized in this study stipulates that the change in the epithelium must be such as to resemble that which is seen in invasive carcinoma. Since there are several varieties of patterns of invasive carcinoma, it follows that there are corresponding varieties of patterns for intraepithelial carcinoma. Even with his wide experience in this area, the cytologist will find truly borderline cases in which the changes appear to be more than atypical hyperplasia and yet still somewhat less than noninvasive carcinoma. For this study the Yates Clinic maintains a panel of three pathologists whose job it is to review such cases.

There are some instances, depending on the judgment of the private physician, when an exploratory laparotomy with possible hysterectomy may be considered if the smear remains positive.

Even after prolonged follow-up, review of original and additional biopsy material and re-examination of the cytologic smears, five of the 100 cases still remain unresolved. Although this is a small percentage, it represents five human lives and therefore is a subject of much concern to the Yates Clinic. So accurate is the diagnosis of cervical cancer by vaginal cell examination that it is the studied opinion of the medical staff of the Yates Clinic that in the case of false positive reports continued follow-up should be resumed until every one has been resolved to the satisfaction of all concerned.

## Conclusion

Use of the vaginal cell examination, popularly known as the "Pap smear", has been proved to be at least 87 per cent accurate as an effective screening technique for detection of cancer of the cervix and therefore should be employed by every practicing physician in the physical examination of all adult women.

A definitely positive cell examination indicates that the patient has cancer. The clinician must then assume the responsibility of proving it beyond a doubt by obtaining a proper biopsy. The most effective way to achieve this is to hospitalize the patient for cold-knife conization and diagnostic curettage. This, in our experience, is the only sure method of obtaining sufficient tissue and of determining the extent of the lesion.

The diagnosis of cervical cancer by vaginal cell examination is so accurate that it is our deliberate conviction that continued follow-up of every false positive report should be resumed until all concerned are satisfied that each and every case has been resolved.

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# Cytodiagnosis of Cancer Using Acridine Orange with Fluorescence Microscopy

*Felix D. Bertalanffy, M.Sc., Ph.D.*

Cytodiagnosis is one of the most readily available and effective means of cancer diagnosis. A small sample of sputum, vaginal or cervical aspirate, or other material, easily collected during routine examination, is all that is needed for diagnosis of the presence or absence of malignant lesions in certain parts of the body.

## Exfoliative Cytology

The clinical use of exfoliative cytology as a method of cancer diagnosis was introduced by G. N. Papanicolaou. It utilizes a general biological phenomenon occurring throughout life in many structures of the body, particularly epithelial. Cells continuously desquamate from the epithelium of the respiratory and digestive tracts, vagina, cervix, endometrium, urinary bladder and other organs and collect in the secretions of these organs. These lost cells are constantly being replaced by new cells. The rates of cell replacement vary greatly, depending upon the type of epithelium; they may range from 2 to 79 per cent of the epithelial cells per day. [1,4,14] Samples of sputum, bronchial secretions, gastric washings, urine, vaginal and cervical aspirates, etc., always contain normal epithelial cells. Together with these cells occur macrophages which usually arise from the subepithelial tissue, as well as leucocytes which have penetrated the epithelial linings.

When malignant tumors arise in epithelial structures, tumor cells likewise desquamate and mingle with the normal cells. Thus, samples of exfoliated material may contain a variety of cells: normal cells, leucocytes and macrophages (in increased numbers in the case of acute and chronic inflammatory processes) and tumor cells. Parasites and bacteria may also occur. Such samples can be smeared on a microscope slide, processed by histological techniques, such as Papanicolaou's procedure, and carefully scrutinized for abnormal cell elements.

## Interpretation of Smears

A thorough knowledge of cytology is necessary for reliable cancer diagnosis. Sometimes malignant cells are very striking, while at other times their distinction from normal or atypical cells is extremely difficult. Some of the main distinguishing features of malignant cells are the following: they may be larger or smaller than normal cells; they may have abnormally large nuclei in relation to the total cell size and these hyperchromatic nuclei may contain unusually large amounts of darkly staining material (DNA). Malignant cells in the same sample may vary greatly in size (anisocytosis), as may their nuclei (anisokaryosis). They may exhibit bizarre shapes which give rise to such terms as "tadpole" and "fiber" to describe the cells.

To recognize with confidence all these malignant changes requires many years of training and experience, and therefore it is usually impossible for the physician himself to make a diagnosis of cancer by means of the technique of exfoliative cytology. However, the high mortality rate in cancer can be effectively lowered only by early detection of cancer. This can be accomplished in two ways: (1) by routine cytodiagnosis in the physician's office and (2) by mass screening programs for large portions of the population.

Many physicians already recognize the

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great urgency of routine cytodiagnosis in their practice, and some of them employ trained cytotechnicians for this purpose. Because of the shortage of reliable cytologists, however, most physicians have to rely upon the services of professional laboratories. The high costs involved in routinely submitting specimens to a cytology laboratory cause many physicians to forego cancer diagnosis by cytologic means. This is unfortunate for patients with malignant disease which might have been detected early by cytologic examination.

This paper describes a new method of cytodiagnosis which enables the physician or his technician, relatively untrained in cytology, to perform prescreening of routinely collected exfoliative specimens. In this way, most normal samples can be discarded, the number of samples to be submitted to a cytology laboratory greatly reduced, and the costs involved in routine cytodiagnosis considerably lessened.

## Fluorescence Microscopy

### Principle

Fluorescence microscopy utilizes the important properties of the dye acridine orange. Acridine orange, when applied to exfoliated cells according to the procedure described below, is a specific stain for the two types of nucleic acid. With this stain, desoxyribonucleic acid (DNA) in the nuclei gives a green fluorescence; ribonucleic acid (RNA) in the cytoplasm fluoresces brown, reddish-brown, orange or bright red, depending upon whether small or large amounts of RNA are present.

This specific histochemical reaction is a manifestation of the fact that ribonucleic acid is closely associated with protein synthesis of the cell. Nondividing or infrequently dividing cells, containing little or no RNA, show a green to brown fluorescence, while cells dividing at physiological rates (e.g., for cell renewal) contain moderate amounts of RNA and give a reddish-brown fluorescence. Malignant cells, which have a very high content of RNA for protein synthesis during rapid formation of daughter cells, fluoresce bright orange to flaming red. In the vaginal epithelium, for example, the metabolically inert superficial cells which have lost most of their RNA fluoresce green, the intermediate cells fluoresce brown, and the basal cells which are the source of new cells by mitosis contain moderate amounts of RNA and hence fluoresce reddish-brown. In contrast, malignant cells have mitotic rates which usually exceed those of the cells of origin and therefore fluoresce orange to bright red because of their much larger RNA content.

### Experience

The fluorescence method, which was introduced by Ludwig von Bertalanffy,[8,9,11] has been tested in close to 25,000 cases. These comprise cases in which there was a high rate of malignancy of the female genital tract,[6,10] specimens consisting of screening material from asymptomatic women in the general population,[6,12] routine samples from the gynecologist's office[3,17] and exfoliated material of all kinds.[2,5,7,13] These investigations have shown that in the hands of the trained cytologist the fluorescence method proves to be as reliable as the conventional techniques for routine cytodiagnosis.

After his own extensive tests, the writer feels confident that the method is a safe, rapid and effective prescreening procedure for the general physician. If the procedure is carried out properly as described below, the number of false negative reports should by no means be higher than that obtained by conventional procedures, even in the hands of physicians with very little knowledge of cytology. However, because a certain percentage of the smears will be suspicious, and under certain conditions normal cells may sometimes show increased fluorescence (see below), the writer strongly advises that a final cytodiagnosis not be attempted by the physician inexperienced in cytology. After sufficient experience, the physician may eventually be able to make the final diagnosis also. The time required to develop this skill will depend upon the amount of material that he has prescreened and upon his interest in exfoliative cytology. The trained cytopathologist should be able to utilize fluores-

cence microscopy for final cytodiagnosis; in rapidly scanning smears, he uses the cytochemical orange-red fluorescence as a "warning signal" and then subsequently ascertains presence or absence of malignancy by studying the morphological criteria which are delineated with great clarity by the fluorescence method.

ADVANTAGES

The major advantage of the fluorescence method is that it enables an untrained observer to readily ascertain whether a smear contains cells which exhibit an orange or red fluorescence and hence may be malignant (or suspicious), or cells which show only greenish, brownish, or reddish-brown fluorescence. The physician or his technician in prescreening smears will thus be able to eliminate most normal samples and submit to a cytology laboratory only those few which are "fluorescence suspicious". The number of specimens thus eliminated may range from 70 to 90 per cent or even more. A second advantage is that the acridine orange may be removed by immersing the smear for some minutes in 50% alcohol, and the same smear, if desired, can then be sent to the laboratory for reprocessing by any of the conventional methods. A third advantage is that only six minutes are required (if fixation is omitted) to prepare a smear for examination with the fluorescence microscope, and only about three minutes for screening. This feature is especially valuable for on-the-spot diagnosis because the physician can reassure the patient after a very short waiting period if the sample proves to be cytologically negative.

## Fluorescence Cytology

No detailed account of the appearance of cells in various samples of exfoliated material under fluorescence microscopy will be given here, particularly because the principle of fluorescence staining is the same regardless of the type of material to which it is applied. It is suggested that the reader study the illustrations and their explanations for more specific details. *(See p. 123.)*

Squamous cells from the oropharyngeal, esophageal, vaginal and vesical epithelia fluoresce green if they are derived from the superficial layers, and brown or reddish-brown if derived from the deeper layers. Columnar cells from the respiratory, gastrointestinal, endocervical and endometrial epithelia normally fluoresce reddish-brown. Mesothelial cells in body fluids also appear reddish-brown. Macrophages fluoresce brown, lymphocytes have reddish-brown cytoplasm and dense, green nuclei, and polymorphonuclear leucocytes are recognized by their green lobulated nuclei and unstained cytoplasm.

Trichomonas vaginalis organisms have brownish-red cytoplasm and small, yellow nuclei. The mycelia and spores of Monilia albicans fluoresce bright red, while bacteria appear red or reddish-brown.

Malignant cells, whether they occur in the respiratory, digestive, urinary or female genital tract, body fluid or other material, are characterized by bright orange to flaming red fluorescence of the cytoplasm and often by greenish-yellow hyperchromatic nuclei.

In some chronic inflammatory conditions, samples of respiratory material and body fluids may contain epithelial cells and macrophages which have an increased red fluorescence of the cytoplasm. In cervical erosions, the endocervical cells may likewise exhibit red fluorescence. Presumably, long-standing irritation stimulates the cells to proliferate at rates faster than normal and therefore they contain more RNA. In spite of their increased fluorescence, such "active" cells are readily distinguished from malignant cells by their normal morphological features. The fact that the occurrence of "active" cells may confuse the inexperienced observer, however, is one reason why the physician untrained in cytology should not make the final cytodiagnosis.

Since acridine orange is a dye which quantitatively demonstrates the amount of RNA in all cells, any increase in the amount of RNA in normal cells will also be shown by the dye. Except for changes in nucleic acid content, there is little cytochemical difference between normal and

malignant cells which can be utilized in a routine procedure. Hence, it is not feasible to develop a specific stain for cancer cells.

## Technique

A fluorescence lamp with a high pressure mercury burner (e.g., HBO 200) and proper filters, which can be attached to any laboratory microscope, is the only equipment required for the microscopy. Such equipment is available at major optical and scientific instrument firms.

The solutions used in this technique keep indefinitely.

Smears are prepared for fluorescence microscopy in the following manner:

(1) Fix smears in a mixture of equal amounts of 95% alcohol and ether. If an on-the-spot diagnosis is desired, fixation is omitted.

(2) Pass rapidly through successive changes of alcohol—80%, 70%, 50%—, distilled water, 1% acetic acid, distilled water. This step requires about one minute.

(3) Stain in acridine orange for three minutes. The dye solution is prepared from 0.10% aqueous stock solution by diluting with phosphate buffer to 0.01%.

(4) Transfer to phosphate buffer (1/15 *M* $KH_2PO_4$ and 1/15 *M* $Na_2HPO_4$ at *p*H 6) for at least one minute to remove excess stain. Smears may remain in this solution for several hours if they are being processed in batches.

(5) Place for one to two minutes into 0.10 *M* $CaCl_2$ to bring about differentiation between RNA and DNA.

(6) Rinse with phosphate buffer, mount coverglass while smear is wet and screen under the fluorescence microscope.

After screening, smears may be destained in 50% alcohol and reprocessed by any conventional procedure. They may be returned to the fixative which also removes the acridine orange, to be stained again later with acridine orange, if so desired. Or they may be dried and stored.

## Summary

The fluorescence method serves a twofold purpose. In the physician's office and in mass screening programs, it can be employed as a fast prescreening procedure, greatly reducing the number of samples that need be submitted to a cytology laboratory for inspection by the cytopathologist. The trained cytotechnician can use the method for the rapid screening of smears and also for the final cytodiagnosis.

## Acknowledgment

The author gratefully acknowledges the kind cooperation of Dr. D. W. Penner, Miss R. McAndrew and Miss J. Trautman of the Department of Pathology, Winnipeg General Hospital, and of Dr. A. M. Goodwin and Mr. F. R. Marks, Manitoba Clinic, Winnipeg, who supplied the cytologic material.

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## Legends

Fig. 1. Macrophages with brownish cytoplasm; some have ingested carbonaceous granules. Sputum. ♂ 78. (X 310.)

Fig. 2. Malignant cells of bronchogenic origin. The cells have a flaming red cytoplasmic fluorescence. They are surrounded by polymorphonuclear leucocytes with green nuclei. Sputum. ♀ 67. (X 310.)

Fig. 3. Respiratory epithelial cells with reddish-brown cytoplasm and green nuclei. Bronchial secretion. ♂ 71. (X 200.)

Fig. 4. Malignant cells from adenocarcinoma, showing red cytoplasm, green nuclei and large red nucleoli. Bronchial secretion. ♀ 57. (X 400.)

Fig. 5. Macrophages (brown cytoplasm) and mesothelial cells (reddish-brown cytoplasm). Note polymorphs with green nuclei and lymphocytes with yellowish-green nuclei surrounded by a narrow rim of reddish cytoplasm. Pleural fluid. ♀ 72. (X 310.)

Fig. 6. Malignant cells with flaming red cytoplasmic fluorescence partially masking the green nuclei. Note conspicuous large red nucleoli. Pleural fluid. ♂ 73. (X 310.)

Fig. 7. Endocervical cells in honeycomb formation, showing reddish-brown cytoplasm and yellowish-green nuclei. Cervical aspirate. ♀ 46. (X 310.)

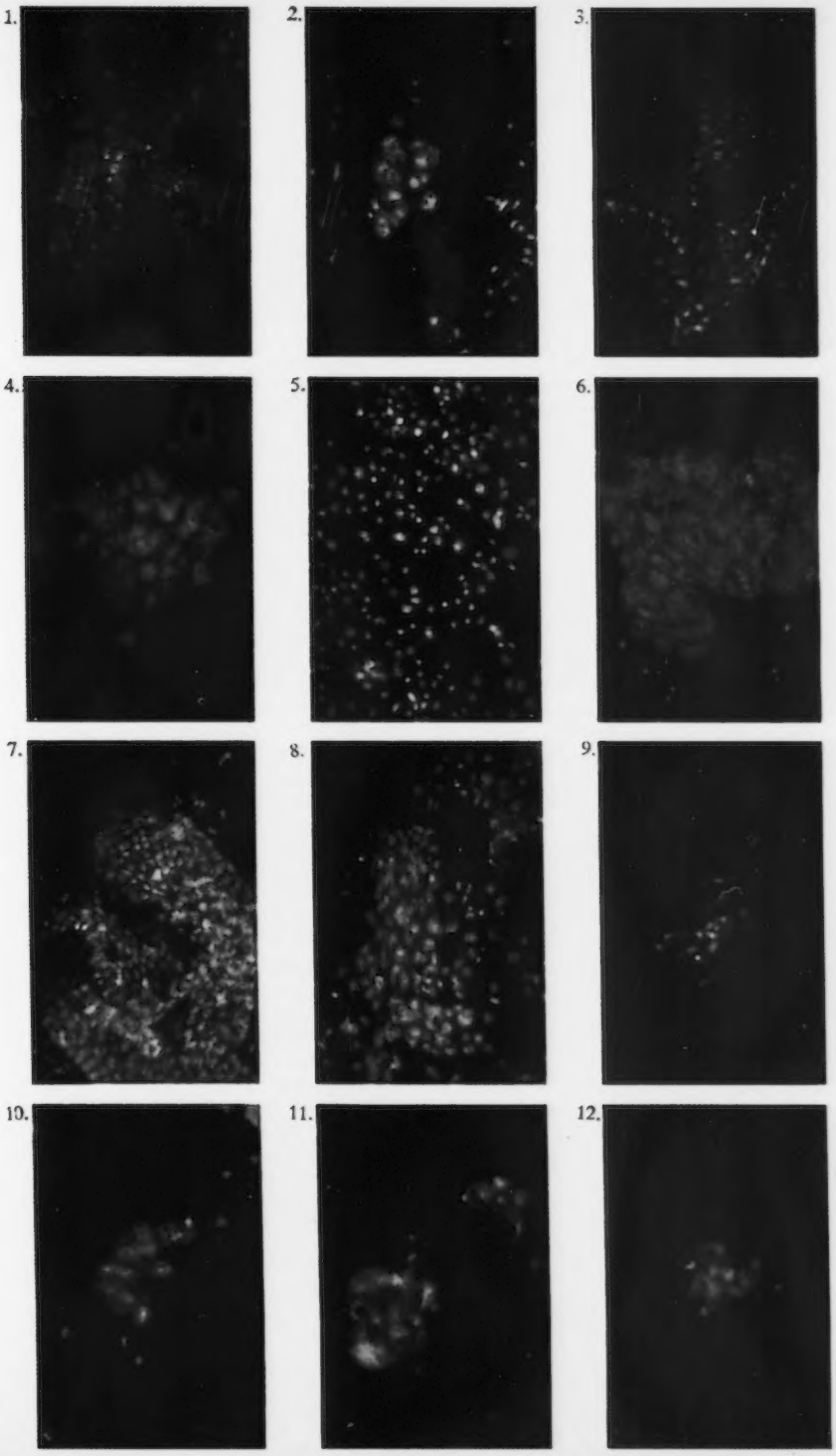
Fig. 8. Malignant cells from squamous cell carcinoma of the cervix, showing bright red cytoplasmic fluorescence, greenish nuclei and red nucleoli. Cervical aspirate. ♀ 62. (X 310.)

Fig. 9. Reddish-brown columnar epithelial cells, partially autolysed. Gastric washing. ♀ 60. (X 310.)

Fig. 10. Malignant cells from adenocarcinoma of the stomach, exhibiting orange-red cytoplasm and large green nuclei. Gastric washing. ♀ 67. (X 500.)

Fig. 11. Malignant cells from adenocarcinoma of the ovary, showing flaming red cytoplasmic fluorescence which partially obscures the yellow nuclei. Ascitic fluid. ♀ 58. (X 310.)

Fig. 12. Malignant cells from squamous cell carcinoma of the bladder, showing intense red cytoplasmic fluorescence and yellow nuclei. Urine. ♂ 74. (X 310.)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# Exfoliative Cytology of the Prostate Gland

Hannah Peters, M. D.

The early diagnosis of carcinoma of the prostate still presents a challenge. The gland, hidden from view, is difficult to evaluate objectively and the examiner has to depend on indirect means to gain an impression of the state of the organ. Processes going on in the center of the gland must remain undetected by digital examination until they have reached the periphery. It is therefore of particular interest to explore the cytologic method which may give information about the epithelium of the gland itself. The cytologic examination of the prostatic smear has, in fact, become a useful method of studying certain diseases of the prostate and is being used effectively as an additional means in the diagnosis of carcinoma of this organ.[3,6]

## Method

To obtain satisfactory preparations, material is collected by prostatic massage. The expressed secretion that collects at the meatus is placed directly on a glass slide on which a thin film of albumin has been spread to insure adherence. A second albuminized slide is put on top of the first and the two are pulled apart. Both slides are immediately immersed in alcohol-ether, fixed adequately and then stained by means of Papanicolaou's technique.

## Cells in Benign Prostate Smears

Very few cells are shed by the young, healthy prostate. A smear prepared from a young man, whose prostate is free from infection, usually contains only an occasional cell which may be easily overlooked on a casual microscopic survey under low power, the cells being of small size (5 to 6 microns). Closer investigation of such a "scant smear" under high power shows the single cells to be *columnar*. With advancing age even the normal prostate desquamates columnar cells freely. A smear from an elderly man without prostatic disease contains many columnar cells (the abundant smear) in loose groups or in small sheets. If the cells occur singly, they are usually well preserved and show a distinct cell membrane (Fig. 1) which cannot always be recognized in cells seen in groups. Their clear cytoplasm is acidophilic, the nuclei are usually larger than those of cells seen in the scant smear and they commonly measure 6 microns, although their size may vary from 3 to 9 microns. They stain lightly and uniformly blue. The nuclear membrane stains deeply, thereby accentuating the pale nucleus. When seen in groups, these nuclei show a remarkable uniformity in size and staining quality.

Columnar cells are seen in large numbers in smears prepared from men with prostatic hypertrophy. Though the desquamation is often profuse and large sheets and dense groups of cells may be found in the smear, there is no pathognomonic cell type or cell change which characterizes this condition.

The *histiocytes* in the prostatic smear, as in other media, appear in a large variety of sizes and shapes. In their most characteristic appearance they form syncytial groups (foreign body giant cells). These round or oval bodies in which the cell membranes have disappeared show abundant cytoplasm. Faintly defined lines and vacuoles are seen in the acidophilic ground substance. The number of nuclei con-

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tained in these structures varies considerably; if there are only a few, they are arranged peripherally and this formation has a very characteristic appearance (Fig. 2). However, when many nuclei are crowded into a single giant cell, they may overlap and appear hyperchromatic. Such a group should not be mistaken for a cluster of malignant cells. More frequently the histiocytes appear as single cells which stand out most clearly from their surroundings. Their size is variable (5 to 10 microns). Their pale pink cytoplasm is often foamy or has large vacuoles. The nucleus, often eccentrically placed, is most easily identified when it is indented. It may stain faintly or heavily and contain a coarse or fine chromatin structure. One or more small nucleoli (their size rarely exceeding 1.3 microns) may be prominent. Histiocytes are seen in large numbers in smears obtained from patients with prostatitis (Fig. 3). Here they mix in dense groups with columnar cells in varying stages of degeneration. Leucocytes, many of which may be eosinophiles, and erythrocytes become part of the cell population. In such smears the marked variability of the histiocytes might add to the difficulty in identifying the cell population correctly as coming from an inflammatory and benign lesion.

*Superficial squamous cells*, desquamated from the epithelium of the urethra or bladder, are only occasionally found in the normal smear obtained by massage. They are polyhedral cells (about 25 microns in diameter) which are occasionally found in small groups (Fig. 4).

*Corpora amylacea* (prostatic concretions) are found in the prostatic smear. Their identification presents no problem.

### Cells in Seminal Vesicle Smears

Quite frequently, smears obtained by massage contain none or few of the cells described so far. Such smears are crowded with spermatozoa and contain cells which are not found in the prostate. These specimens are apparently obtained from the ampulla of the ductus deferens by stripping the seminal vesicles in the course of the massage.[4] Their cell population consists mainly of spermatozoa which may be distributed so densely as to obscure all other cells. In some preparations spermatids are seen among the spermatozoa. These are very small cells (8 microns) with a uniform, deep staining, eosinophilic cytoplasm. An eccentrically placed nucleus, which reveals no chromatin detail, gives the cell a characteristic appearance. Spermatids with two nuclei located at opposite poles of the cell, or with three nuclei in a clover-leaf arrangement, as well as cells with multiple nuclei arranged around the cell periphery, can be recognized on high power survey. These cells are of interest in sterility problems because an increase in sperm precursors can be related to male infertility.[2]

There are usually many epithelial cells in a seminal vesicle smear which are often bizarre and may show marked variation and atypia. Some of them contain a yellow pigment, but unfortunately the pigment is not a constant finding which would identify these cells simply and definitely as coming from the lining of the seminal vesicles. Many histiocytes are always present which contain yellow pigment or, more frequently, engulf varying numbers of heads of spermatozoa. If the heads are only few, the cell will be recognized easily as a histiocyte. However, if the histiocyte is densely packed with fragments or whole heads of spermatozoa, then these dark blue masses may be mistaken for chromatin bits of a hyperchromatic nucleus. Caution should be exercised in diagnosing abnormal cells in a seminal vesicle smear also because of the marked atypism of epithelial cells lining the normal seminal vesicles. If groups of cancer cells are seen in a seminal vesicle smear, a repeat smear should be made after a massage that avoids the seminal vesicles. It is desirable to find the abnormal cells in the medium coming directly from the prostate in order to avoid error in diagnosis.

### Cells in Prostatic Cancer Smears

Cancer of the prostate sheds cells freely and massage will produce ample material

if the ducts are patent. The cancer cells are seen in groups and clumps, large sheets and dense cast-like structures. There is usually marked crowding and overlapping of nuclei. Cell membranes are not recognized, especially in the crowded portions of groups. The cytoplasm is sparse, as a rule, and in some groups large cytoplasmic vacuoles are striking. The nuclear membrane is thick and distinct; marked anisonucleosis, hyperchromasia and irregular chromatin distribution characterize the nuclei which may contain multiple and large (2 to 3.5 microns) nucleoli. Not all of these characteristics are present in each group of malignant cells; one or more may be absent. Cancer cells seen singly or in loosely arranged groups show some of the characteristics usually with great clarity (Fig. 5).

Other cells will be intermingled with the cancer cells in the smear, their number and kind depending on the admixture of secretion from parts of the prostate other than that harboring the cancer. Groups of columnar cells will be shed from normal and hypertrophic parts of the gland. If infection is present, inflammatory cells will be seen. Histiocytes, leucocytes and erythrocytes often form a considerable part of the cell population in carcinoma.

## Cell Changes After Estrogen Therapy

Estrogen therapy produces marked changes in the cell population of the prostatic smear. These are characteristic and can be used as a guide in the hormone therapy.[5] By following these changes in repeated smears it is possible to judge the response of the cancer and estimate its hormone sensitivity. The changes which are noted soon after the patient begins the therapy become evident in the cancer as well as in the cells shed from other parts of the prostate.

Shortly after hormone therapy has been started, a profuse desquamation of benign cells begins. The most striking feature of a smear from a patient on estrogen therapy is the abundance of *large superficial squamous cells*. This cell, which is only rarely part of the cell population before hormones are taken, becomes the main cell type in the smear. To the casual observer, smears in which the cancer cells have disappeared after prolonged hormone therapy look therefore not unlike vaginal smears. The large polygonal cells stain intensely acidophilic; they are flattened out and often contain round, basophilic cytoplasmic granules.

A new cell type, not otherwise seen in the smear, begins to appear after hormone therapy. The *glycogenic cell* is a most characteristic cell found while the patient continues to take estrogen (Fig. 6). It is desquamated from areas of metaplasia which develop in the prostate stimulated by hormone treatment. Glycogenic cells are found in smears not only from patients with carcinoma of the prostate, but also from all patients taking estrogens for any reason. They occur in the smear as early

## Legends

Fig. 1. Single columnar cells with well preserved cell membrane. (X350.)

Fig. 2. Foreign body giant cell with peripherally placed nuclei. (X350.)

Fig. 3. Group of columnar cells and histiocytes with foamy cytoplasm. Patient with prostatitis. (X350.)

Fig. 4. Cells desquamated from transitional epithelium. (X350.)

Fig. 5. Loose group of cancer cells with thick nuclear membranes and large nucleoli. (X350.)

Fig. 6. Large group of glycogenic cells after 10 weeks of estrogen therapy. (X150.)

Fig. 7. Group of "altered" malignant cells and many superficial squamous cells. Four glycogenic cells are in the field. Smear of a patient with cancer of the prostate after estrogen therapy for two months. (X350.)

Fig. 8. Large superficial squamous cells and glycogenic cells in a smear of a patient whose cancer responded to estrogen therapy. Malignant cells were no longer seen in the smear. (X350.)

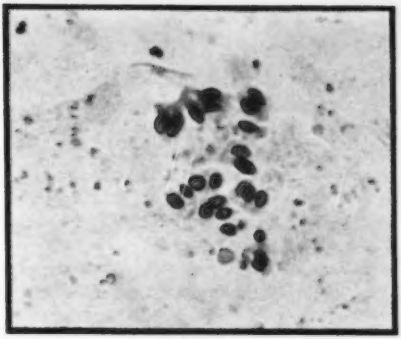

Fig. 1

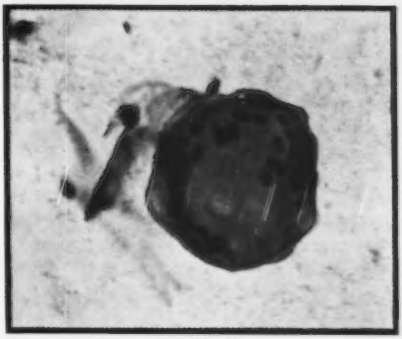

Fig. 2

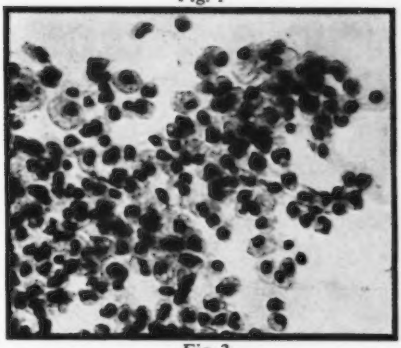

Fig. 3

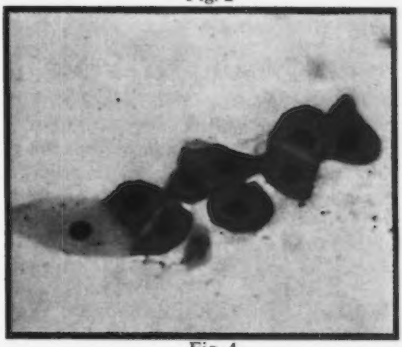

Fig. 4

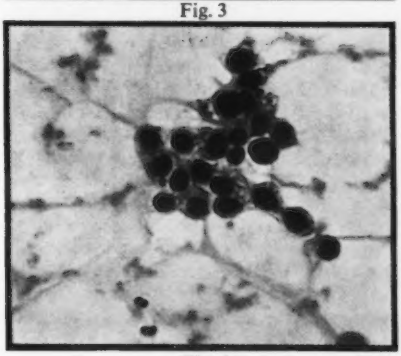

Fig. 5

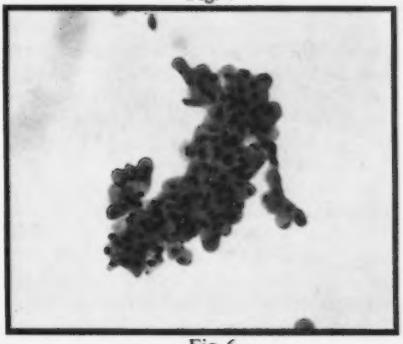

Fig. 6

Fig. 7

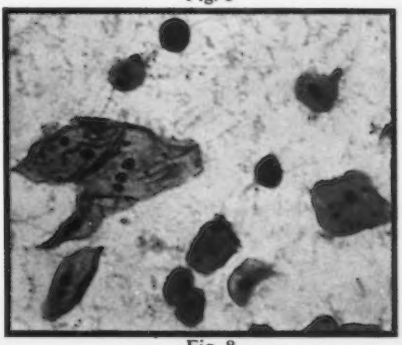

Fig. 8

as a few days after therapy is begun and promptly disappear when the treatment is discontinued. Their presence or absence is a sensitive indicator as to whether or not the patient has continued his therapy. A casual survey under low power magnification suffices to recognize them. They are round or oval in shape and appear singly or occasionally in large groups. A dense peripheral rim of cytoplasm and an empty central portion are characteristic. Specific stains show that this central part contains glycogen.

### Changes in Cancer Cells

The characteristic features of cancer cells which are well defined before estrogen therapy is started soon become indistinct, and identification is uncertain. The clumps of malignant cells appear to retain their outline for quite a while, but details of the nuclei soon become masked and unclear. The nuclei which are still crowded and overlapping begin to shrink, lose chromatin detail and stain uniformly dark. Only the appearance of the whole group characterizes them as *"altered" malignant cells* (Fig. 7). If estrogen therapy is continued, the number of altered malignant cells in the smear decreases and only degenerated cells and cell clumps remain as remnants of the malignant cells. In cases in which the prostatic cancer responds favorably to the hormone therapy, even these remnants disappear altogether and no cells referable to a cancer remain in the smear. In those cases in which the cancer does not respond to hormone therapy, cancer cells continue to be found in the smear unaltered and present their usual characteristics.

It becomes understandable that smears from patients undergoing hormone therapy may have a variable picture, depending not only on the duration of treatment but also on the hormone sensitivity of the tissues. Many smears from patients taking hormones will not show any cancer cells (Fig. 8), although the original diagnosis was cancer. At times such findings have been reported in the literature as false negative results and have been thought to indicate an inadequacy of the smear method in the diagnosis of carcinoma.[1] However, a smear that fails to show cancer cells under these circumstances reveals a sensitivity of the cancer to hormone. The presence of large squamous cells, and particularly of glycogenic cells, indicates a proliferation of the squamous epithelium and the existence of squamous metaplasia stimulated by the hormone treatment. The dynamic cell changes observed in smears taken at frequent intervals can constructively add to the clinical and laboratory evaluation of the progress and response of the disease to therapy.

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# Cytology of the Urinary Tract and Prostate

John F. Seybolt, M.D.

With the aid of such techniques as cystoscopy and intravenous or retrograde pyelography, a high percentage of genitourinary disorders may be accurately diagnosed today. When the presence of a malignant neoplasm can be recognized by one or more of these means, the tumor must already have developed to a size large enough for the examiner to visualize it directly or indirectly; the attainment of this degree of neoplastic growth diminishes the chances for successful treatment. Exfoliative cytology now permits us to study the malignant nature of the lesion while it is still very small, making it possible at times to discover carcinomas of the urinary tract which are still in the early, even preinvasive, developmental phases. It may also be of considerable help in the differential diagnosis of lesions which are inaccessible to biopsy.

The cytologic detection of a malignant neoplasm of this region depends upon the recognition of cancer cells shed from the surface of the tumor and occurring as components of a bodily excretion or secretion, usually either urine or the specimen obtained by prostatic massage. The collection of specimens and even the preparation, fixation and staining of the smears are for the most part simple procedures which may be performed in the office by the general practitioner. However, the acquiring of a specimen by means of special techniques, such as ureteral catheterization, necessitates the help of a urologist. The microscopic interpretation of cytologic findings also requires specialized training and should not be attempted by those who lack the proper experience. Even the well trained pathologist is not necessarily familiar with the wide variations in cellular morphology encountered in urinary sediments. Fixed specimens or prepared slides therefore should be submitted to a reliable cytology laboratory which can validly interpret the smears.

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## Types of Specimens

### Bladder Urine

Being the easiest to collect, bladder urine constitutes the bulk of genitourinary specimens submitted for cytologic study. It may be simply a voided sample in the case of male patients, but in females it should be obtained by catheterization to avoid contamination by desquamated vaginal cells. The amount of specimen desired varies among cytologic laboratories, but an adequate sample will measure not less than 50 ml; smaller volumes are impractical because of the low number of cells usually found. Best results are obtained with even greater quantities of urine, but few laboratories are equipped to handle large volumes routinely.

The first urinary output of the morning is considered preferable; an approximately equal amount of 95% alcohol should be added to the specimen. If laboratory processing is delayed, refrigeration should be used to preserve the cells. To avoid transporting large volumes of urinary specimens to the cytology laboratory, the entire specimen may be left to stand undisturbed for half an hour, the top clear layer then decanted and only the sedimented portion submitted to the laboratory. However, if the settling-out period is excessive-

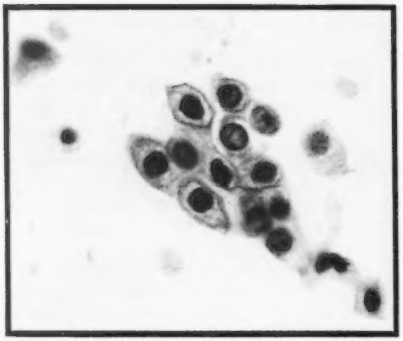

Fig. 1. Normal transitional cells. Voided urine. (X600.)

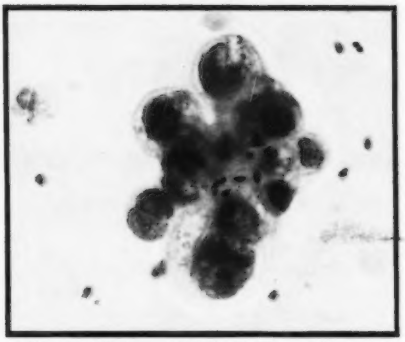

Fig. 2. Malignant cells from another case of prostatic carcinoma. Cells show pleomorphism. Voided urine. (X600.)

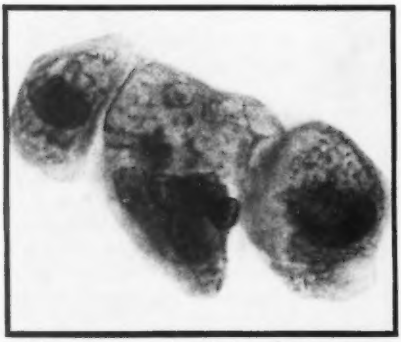

Fig. 3. Group of cells in urine specimen. Note multinucleation of central cell. Urine obtained by ureteral catheterization. (X600.)

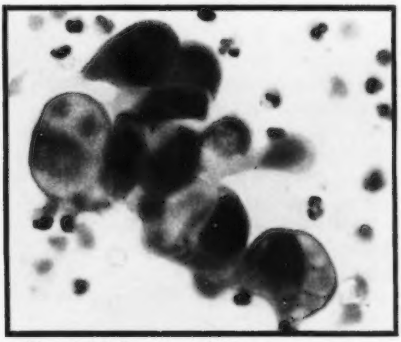

Fig. 4. Malignant cells from a case of carcinoma within a diverticulum of the bladder. Voided urine. (X600.)

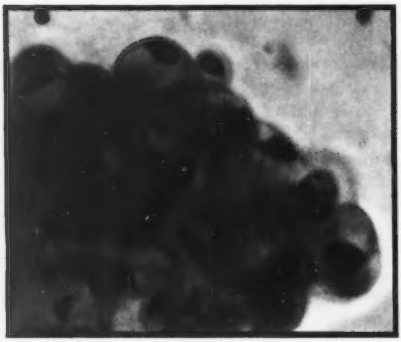

Fig. 5. Malignant cells from a case of prostatic carcinoma. Note benign appearance of nuclei and vacuolation of cytoplasm. (X600.)

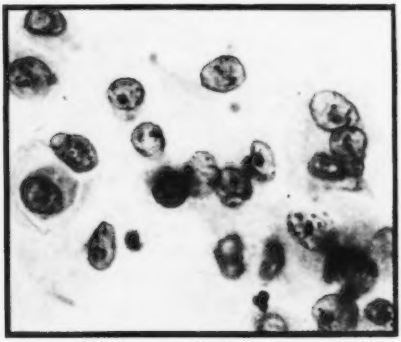

Fig. 6. Malignant cells from a case clinically diagnosed as carcinoma of the kidney. No autopsy was obtained. Voided urine. (X600.)

ly prolonged, a fixative is used to prevent growth of contaminating bacteria and cellular degeneration.

Urinary specimens not infrequently contain so few cells that the cytologist is inclined to report them as "inadequate for cytologic evaluation". Laboratory methods of concentrating cells will be considered later on, but one maneuver which should be mentioned here, because it has been found to increase the number of cells in a specimen, is to have the patient exercise prior to voiding.[12] The agitation of urine against the bladder wall apparently produces a richer exfoliation of mucosal cells. This innovation, although perhaps useful in connection with the diagnosis of vesical neoplasms, would presumably do little to enhance the quality of specimens for detection of tumors in other locations. One wonders if a similar effect might not be produced safely by gentle massage of the suprapubic region in bedridden patients or others whose activity is restricted.

### Ureteral Urine

When tumors of the ureter, renal pelvis or kidney are suspected, the urine specimen of choice is that obtained by ureteral catheterization. Obviously, the side from which the sample is collected should be carefully noted on the container and on the request form submitted to the laboratory. The amount of ureteral urine should be as large as is feasible, but at least 10 ml is ordinarily required. The addition of an equal volume of 95% alcohol is recommended as a preservative.

### Prostatic Massage Specimens

Prostatic secretions may be obtained in the same manner as they are for bacteriological examination, the expressed secretion being collected on a glass slide held at the urethral meatus. Some workers prefer that the slide be coated in advance with a thin film of albumin to assure adhesion of the cells to the slide during fixation and staining. Fergusson and Gibson believe that the method of expressing prostatic fluid is extremely important and they recommend a procedure based on anatomical and physiological considerations. In their opinion, the secretion from the apex (inferior pole) of the gland is the most informative because this portion of the gland, which is usually hyperplastic, is composed largely of the prostatic elements in which carcinoma is most likely to develop. The results of their investigations in detecting prostatic carcinoma were so encouraging that it is perhaps worthwhile to describe their method of expressing fluid.

The patient is placed facing the examination couch with his legs apart, body bent forward at the hips, steadying himself with one hand. With the examiner's finger in the rectum, massage is begun as far forward as possible on one side of the gland at the level of the apex. Pressure is applied as firmly as the patient will allow and the finger slid posteromedially toward the median groove. This movement is repeated several times at the same level on both sides of the gland. Similar massage is then performed at successively higher levels, finishing at the base (superior portion) of the prostate. One must avoid massaging the seminal vesicles in order to prevent contamination by cells from that source. When the massage is completed, the finger is gently pressed over the urethra from above downwards, following through as far as possible onto the bulbous urethra. At the same time further stripping may be continued by exerting sliding pressure with the thumb on the perineum until the required amount of fluid is produced at the external meatus.

When successive drops of secretion have been obtained on several prepared slides, a fresh, clean slide is placed on top of each preparation and the pair of slides pressed lightly together. The coherent slides are then gently slid apart, producing a thin film of secretion on each one. The authors conclude that cytologic examination of this type of prostatic smear offers a reliable method of estimating the condition of the gland with regard to malignancy, giving results comparable to those of biopsy without the attendant risks.

It is important not to permit more than minimum drying around the edges of the

smear before placing it in a solution of equal parts of ether and 95% alcohol. Further drying leads to distortion of the cells and interferes with their proper staining, making microscopic evaluation difficult or impossible. Fixation should continue for at least 15 minutes, although the slides may be left in the fixative for longer periods of time.

In regard to the use of massage for obtaining prostatic smears, the question is frequently asked: To what extent does massage encourage spread of the disease in a patient with prostatic carcinoma? This question has not been answered by any authoritative study to date, but Albers, McDonald and Thompson state: "It is hard to conceive that massage would spread carcinoma more than does transurethral resection or aspiration biopsy, and there is no evidence in the literature that spread by those procedures occurs." If cytologic studies are to be performed at all in cases suspected of prostatic neoplasia, some massage will generally be required to provide the cells which form the basis of an accurate cytologic report. It will be shown later that urinary specimens collected without massage are usually of little value in the diagnosis of prostatic carcinoma, except in advanced cases.

The stony hard prostate, itself suggestive of carcinoma, and the rubbery, thickened prostate of adenomatous hyperplasia within which a neoplasm might be concealed, may fail to yield sufficient material on massage; also, an inflamed prostate may be too tender to permit adequate manipulation. Several investigators, however, have been able to obtain satisfactory specimens in a sufficient percentage of cases to make it worthwhile to attempt to arrive at the diagnosis of prostatic cancer through exfoliative cytology. The actual results to be expected will be discussed below.

A urinary sample collected immediately after prostatic massage, particularly in those cases in which meager quantities of secretion have been obtained, may prove to be a valuable specimen cytologically. This procedure is especially useful when the prostatic fluid expressed by massage reaches the lumen of the prostatic urethra but is of too small a volume to attain the urethral meatus.

### Semen

The cytologic examination of semen may be useful in some instances for suggesting the neoplastic nature of a disease process in the testis or epididymis, although the actual results of this type of examination of specimen have not yet been adequately determined. As in the case of prostatic smears, the ejaculate is spread thinly on glass slides and fixed immediately (before drying) in equal parts of ether and 95% alcohol for at least 15 minutes.

## Laboratory Processing

Specimens of prostatic secretion and of semen are already prepared on glass slides and fixed when they are received in the laboratory. The staining procedure is the same as for smears of urinary sediment.

Urinary samples are centrifuged in the laboratory as soon as possible and the supernatant discarded. The entire residue is smeared on three or more glass slides coated with albumin, and fixed in a mixture of equal parts of ether and 95% alcohol. After fixation for at least 15 minutes, submersion for one-half minute in a 1% solution of celloidin in alcohol-ether is helpful to assure adherence of the cells to the slide during the staining procedure which is done according to the Papanicolaou technique.[16] This method of processing urinary samples constitutes the standard method still widely used. There have been several attempts to improve it, however, principally with regard to increasing the cellularity of the smears. Some of these will be described briefly.

Deden utilized relatively large volumes of urine (400-500 ml) and permitted the sedimentation of the solid elements by means of separating funnels. The sediment was then centrifuged. The greater number of cells obtained per microscopic field was found to be advantageous for cytologic interpretation. A similar, though perhaps less striking, effect may be produced by

simply allowing the urinary specimen to stand for one-half hour. After decanting and discarding the superficial clear layer, the sedimented portion is centrifuged. With this latter procedure it has been shown[11] that the average number of cells per microscopic field may be increased twofold.

The use of the Millipore filter has recently been advocated[24] as a means of increasing the cellular content of microscopic preparations of urine. Filtration through the cellulose membrane with pores 5 microns in diameter is aided by a vacuum pump operating at a negative pressure of 25 mm Hg. Large volumes of specimen are rapidly passed through the filter which withholds the cells and which is then mounted on a glass slide and stained. The use of this apparatus for cytologic study was initially suggested by Seal who found it helpful in his investigations on serous fluids.

Another technique is that described by Sagi and Mackenzie which is also purported to increase significantly the cellular concentration of preparations. A 200 ml sample of urine is distributed in centrifuge tubes after having been cleared of urates, phosphates, carbonates and erythrocytes. After centrifugation, the supernatant is discarded and the residue from all tubes combined into one tube, a small amount of distilled water being used as the vehicle. This tube is centrifuged again and the supernatant discarded. One or more drops of a mixture of equal parts of cell-free saliva and glycerine are stirred into the sediment to increase its cohesive properties; this suspension is then transferred by pipette to a single glass slide and spread evenly over its surface. Fixation is accomplished with absolute acetone and the Papanicolaou staining method used.

## Cytologic Interpretation

### Urine

NORMAL SMEARS.—Although urinary samples may normally contain small numbers of cells from the epithelium of other portions of the genitourinary tract, such as the seminal vesicle, prostate, etc., most of the cellular components are derived from those parts of the tract lined by transitional epithelium—renal pelvis, ureter and bladder. These exfoliated transitional cells assume many diverse forms when seen in the fixed and stained preparations, the greatest diversity being found in specimens obtained by ureteral catheterization. The cells vary markedly in size and shape, number of nuclei and nuclear morphology. The variations range from small cells with single nuclei to huge cells with 80 or more nuclei, all being within normal morphological limits.

To the student of cytologic interpretation such an array of cellular forms presents a discouraging task in his attempt to familiarize himself with what can safely be considered "normal". This feature is not encountered to such a degree in any other application of exfoliative cytology. Considerable practice in the examination of urinary sediments is required before one feels reasonably secure in his evaluations, even though his experience may have been extensive in, say, vaginal or pulmonary cytology.

ATYPICAL SMEARS.—Nonmalignant conditions which may affect the morphology of exfoliated cells in urinary specimens include inflammation (when, in addition to alteration of some of the epithelial cells, there may be numerous polymorphonuclear leukocytes or lymphocytes), calculi anywhere in the tract, papillomata, changes due to radiation therapy, metaplasia and changes due to hormonal therapy. It is not always possible to recognize cellular atypia as being attributable to any one of these conditions, but at times the changes are suggestive of a specific condition. In most instances there will be little confusion between these changes and those due to malignancy, but the worst offender seems to be the presence of calculi which have led to false positive cytologic interpretations upon a few occasions.

HORMONAL EFFECTS.—Urinary sediments have been shown to reflect hormonal effects in women, in both the pregnant and nonpregnant states.[13, 17] Endometrial histologic changes during the men-

strual cycle have been closely correlated with urinary cytologic patterns.[25] Also, men receiving estrogenic therapy for prostatic carcinoma show alterations in the cells from the urinary tract and prostate.[18] The application of urinary cytology to the diagnosis of pregnancy, although of some help, probably has limited usefulness, for it was found by McCallin *et al.* to be least reliable during the early periods of gestation when such additional information would be particularly desirable. Typically, urinary smears taken during pregnancy show a heavy cellularity with superficial and intermediate cells in about equal numbers. The cell type most helpful in the determination of pregnancy is the navicular cell which is elongated, spindle- or boat-shaped, has a heavy, doubly refractive boundary and is generally smaller than the intermediate cell of the nonpregnant state. The nucleus may be larger, compact or pyknotic, elongated, and often is flattened against the periphery. Apparently, these features are the result of the effect of estrogen and progesterone combined rather than of estrogen alone.

Men receiving estrogenic therapy for prostatic cancer show changes in their urinary smears similar to those found in women taking estrogens for postmenopausal symptoms. The smears are characterized by heavy cellularity, increased number of superficial cells, pyknosis, eosinophilia and cytoplasmic granules. The most striking difference between these smears and those of pregnancy urine is the lack of navicular cells and the marked eosinophilia in the smears of patients receiving estrogenic hormones.[13]

MALIGNANT CELLS.—In the cytologic diagnosis of malignant neoplasms, the nuclear alterations constitute the principal criteria. The changes to be looked for include nuclear enlargement, pleomorphism, increased chromatin, irregular chromatin clumping, prominent nucleoli, etc. Cytoplasmic criteria are also helpful at times but are not nearly so important. The cytoplasmic staining reaction plays only a relatively minor role.

Whereas in the cytology of the female genital tract cellular forms and patterns are sometimes found to be suggestive of an *in situ* lesion of the uterine cervix (the so-called "dyskaryotic pattern" of Papanicolaou), the abnormal cells found in urinary samples from cases of carcinoma *in situ* of the bladder have not been distinguishable from those obtained from more advanced lesions. Most of the carcinomas whose exfoliated cells are found in urinary specimens are of the transitional cell type, with squamous cell carcinoma running a close second. Adenocarcinomas of this tract are not frequently encountered except in the prostate. Carcinoma of the kidney (renal cell carcinoma, hypernephroma) is usually in an advanced state when the urine contains cells from this source since the tumor must of necessity have eroded through the walls of the collecting system before its cells can gain access to the urinary passages. Another difficulty in regard to cytologic diagnosis of hypernephroma is that the component cells are rather benign in appearance when they are examined individually. However, the cytologic detection of renal carcinoma has been and is being accomplished, though usually late in its course of development.

### PROSTATIC SECRETION

Columnar epithelial cells, transitional and squamous cells, histiocytes, prostatic concretions (corpora amylacea), lymphocytes, polymorphonuclear leukocytes and spermatozoa comprise the principal cellular elements found in normal prostatic secretions.[19] When prostatic carcinoma is present, desquamated cancer cells also may be found. Mulholland, in 1931, contributed to the early studies of prostatic secretions as applied to the diagnosis of cancer. In general, the morphologic features leading to cytologic diagnosis of prostatic carcinoma are similar to those found in cancers of other organs. However, accurate appraisal here is dependent more upon the finding of clusters of cells than of dispersed single cells, for frequently the degree of cellular pleomorphism is not nearly so pronounced as in many other types of neoplasm and the cells are often relatively small. The identification of malignant cells

is at times quite simple for the trained observer, particularly when there is a marked degree of variation in nuclear morphology. In the case of a well differentiated carcinoma, however, the evaluation may be considerably less certain since the cells comprising such tumors may be very difficult to distinguish from the normal.

Peters and Benjamin have described changes which occur in malignant prostatic cells after treatment with estrogens. These so-called "altered" malignant cells showed uniformly dark-staining nuclei with indistinct, irregular nuclear outlines and loss of chromatin detail; the cytoplasm was very scant or absent. Such changes may cause uncertainty in the recognition of the malignant nature of the cells. Continuous estrogenic therapy was found to result in the disappearance of these "altered" malignant elements, and only degenerated cells and cell clumps were seen. In those cases in which the clinical response to estrogen therapy was favorable, malignant cells disappeared from the smears altogether. Conversely, in cases in which malignant cells persisted in the smears, a poor clinical response to therapy was noted.

## Results to be Expected from Cytologic Studies

### Urinary Tract

As mentioned above, one of the principal values of the cytologic examination of genitourinary material is that it lends itself admirably to the detection of malignant lesions while they are still in early stages of development. It is also helpful in bringing to light the presence of malignant neoplasms located in places not readily accessible to cystoscopic examination or other diagnostic techniques. For example, Foot and Papanicolaou reported a case of early renal carcinoma *in situ* which was discovered through cytologic studies. In this instance, urine from the bladder of a 70-year-old white woman was reported as Class IV, and urines from the right ureter on two separate occasions yielded Class V reports. Urine specimens from the left ureter provided only Class I and II reports. Cystoendoscopy and intravenous and retrograde pyelography were normal, even in retrospect, but blood was seen coming from the right ureteral orifice on one occasion. After nephrectomy, the kidney specimen showed no gross evidence of tumor, but many microscopic sections from four calyces demonstrated carcinoma *in situ* in three of them. The authors state: "It is noteworthy that there were many clusters of cancerous cells either detached or in the process of desquamation from the surface of the tumor. It is such clusters that appeared in the smears of the urinary sediment, and the profuse desquamation of cancerous cells observed in this case is, therefore, strongly in support of the idea that carcinomas *in situ* do indeed shed their elements freely. This feature is pregnant with diagnostic possibilities in the case of carcinoma *in situ* in inaccessible areas of the body."

In our laboratory there has also been at least one instance in which a small carcinoma, arising within a diverticulum of the bladder and thereby escaping detection by the cystoscopist, was found after examination of the urine showed the presence of malignant cells.

Harrison *et al.* point out too that cytology is useful in the earlier detection of recurrence after conservative surgery for tumors of the bladder. In line with this, one of the patients from whom specimens had been submitted to our laboratory was a 53-year-old white woman with a history of four previous transurethral resections for proved papillary carcinoma of the bladder. Her urine had been reported as Class V (definitely positive) on numerous occasions in the past. At a time when no evidence of urinary cancer was noted upon careful examination, another Class V report was issued. Visualization by cystoscopy two months later revealed no bladder tumor, but biopsy of a slightly erythematous area revealed papillary carcinoma.

For the purpose of early detection of any cancers of the bladder that might develop as a result of contact with dyestuffs, Crabbe *et al.* examined urinary sediments of 1,800 men who worked with these compounds in their occupation. They reported

that 67 men had positive smears and 51 showed tumors of the bladder on their first cystoscopic examination. Five others who had positive smears were examined cystoscopically and no tumor was demonstrated, but all five men developed tumors of the bladder five months to four years later. Another man who had positive smears was found to have clinically demonstrable prostatic carcinoma. Tumors were discovered, therefore, in 57 (85.1 per cent) of the men with positive smears. Smears of the remaining ten were regarded as false positive. Crabbe and his co-workers concluded: "The most important advantage of the method is its ability to indicate the presence of a tumor at a very early stage of its development, often before any presenting symptoms or other urinary abnormalities have given an indication of its presence."

Foot *et al.*[7] analyzed a series of cases, mainly from the New York Hospital, the urinary specimens of which were submitted for cytologic examination. In 678 cases involving disease of the bladder, renal pelvis and ureter, there were 212 cases harboring cancer which arose in transitional epithelium. Of these 212, 168 (79 per cent) were regarded as suspicious or positive for cancer, on the basis of the cytologic findings. Smears from the remaining 21 per cent, on the other hand, were interpreted as negative. It should be pointed out that negative diagnosis means but one thing: no cancer cells were found; there may have been none to find because exfoliation may not have occurred; their numbers may have been so minute that these cells were missed in the course of examining several smears in a set, or their significance may have gone unrecognized. Thus, a Class I or II diagnosis by no means implies that the patient is free from cancer; it merely indicates that the presence of cancer is unproved. In the group of 466 patients who did not have cancer, the specimens were correctly interpreted as negative in 90.8 per cent; they were suspicious in 8 per cent, and false positive in 1.2 per cent.

Roland and Marshall investigated the reliability of 442 positive urinary cytologic reports made on 176 patients in another series at the New York Hospital. They found that all but eight of the patients in whom positive cytologic reports were rendered proved to have cancer. Therefore, in 95 per cent of the cases with Class IV and V interpretations, the cytologic diagnosis of cancer was correct. When the slides from the eight false positive cases were reviewed, only two were still thought to warrant positive evaluations. Five of these eight patients were followed for five years or more without a diagnosis of cancer ever being made; two died within five years without evidence of cancer, and one patient was lost to follow-up. The authors state that the group of eight unexplained, cytologically positive cases out of a total of 176 patients with Class IV or V urine reports is sufficiently small to justify a high suspicion that a patient with a positive cytologic report harbors in his urinary tract a cancer which may not yet have made itself evident in the usually recognizable form.

McDonald found that in 78 cases in which lesions of the bladder were suspected, only 36 showed cancer cells in the sediments while the remaining 42 had negative smears. Three (8 per cent) were false positive. Harrison *et al.*, however, reported finding cancer cells in all of 67 cases of carcinoma of the bladder, while Deden detected cancer cells in 62 (85 per cent) of 73 cases of proved cancer of the urinary tract. Of 50 patients with carcinoma of the bladder in Deden's series, 46 (92 per cent) had positive smears. Chute and Williams found cytologic evidence of malignant disease in 32 (54 per cent) of 59 patients with cancer of the genitourinary tract. Twenty-nine patients had carcinoma of the bladder; in 65 per cent the cancer was detected by smear.

### Kidney

It is quite obvious that intrarenal tumors lying within the parenchyma of the organ do not shed cells into the urinary passages (e.g., the renal pelves) until they have penetrated the walls of these structures. Therefore it should not be anticipated that tumors of this type will be discovered as

readily as vesical neoplasms through exfoliative cytology.

In their group of 543 patients with diagnoses pertaining to the kidney, Foot *et al.*[7] found that 60 were proved to have carcinoma, 33.3 per cent of whom were given suspicious or positive cytologic reports. In 483 cases of renal disease in which no cancer was found, there were five (1 per cent) false positive reports. Harrison found cancer cells in 12 of 15 cases of carcinoma of the kidney, and in Deden's series of 15 cases of renal carcinoma, malignant cells were found in the smears of 11. Chute and Williams gave a positive report in one of three cases of renal cancer.

### Prostate

The validity of cytologic studies on prostatic secretions was shown by Fergusson and Gibson to be high. In their hands, 94.7 per cent of 40 cases with prostatic cancer yielded recognizable malignant cells in the smears, while 96.4 per cent of 55 cases without cancer produced smears which were correctly read as negative. The technique they used for thoroughly "milking" the gland has been described above. Their results suggest the necessity of resorting to this special method of collecting prostatic specimens if success in the cytologic detection of carcinoma is to be realized.

In the series reported by Foot *et al.*, urine specimens were received from most of the patients with prostatic disease. Only 36 per cent of the 95 cases with prostatic cancer were given positive and suspicious cytologic reports. This result emphasizes the undesirability of urinary specimens for cytologic study in cases of suspected prostatic cancer. Herbut found cancer cells in the prostatic secretions of 54 patients (81.8 per cent) in a series of 66. There were three false diagnoses of cancer. He recommended this method as a routine diagnostic procedure. Peters and Young studied the prostatic smears of 44 patients with carcinoma of the prostate and found malignant cells in the secretions of 38 of them, or 86.4 per cent. Boyer, on the other hand, was able to detect carcinoma of the prostate by cytologic means in only 16 cases out of 44, or less than 40 per cent. He offers the suggestion that perhaps these poor results may be attributable to the fact that most of his patients were under estrogenic therapy at the time of examination. Furthermore, the advanced state of the neoplastic process in many of them also may have played a role; some of the glands were either so indurated or tender that prostatic massage was quite inadequate and the amount of secretion expressed was minimal. Of Deden's six cases with carcinoma of the prostate, three had positive smears. There were 24 cases of prostatic carcinoma in the series reported by Chute and Williams, 42 per cent of which gave positive smears. Half of their patients had been receiving stilbestrol, and one had undergone orchiectomy.

## Pitfalls in Evaluating Cytologic Findings

False negative reports probably constitute the largest single group of cytologic failures. "False negative" means that the cytologic report gives no indication of malignancy in a case known to harbor a cancer. There are several explanations for this type of error. Exfoliation of cancer cells may have been prevented because of the position of the tumor; for example, the tumor may lie deep within the renal parenchyma where there is no communication with the urinary passages. Even if its position is intraluminal, as in the case of papillary carcinoma of the bladder, the surface of the tumor may be so necrotic that there is no shedding of well preserved malignant cells. No other explanation seems to be so likely for the few instances in which negative reports were issued in spite of the presence of rather large carcinomas of the bladder. Neoplasms comprised of well differentiated cells may also be interpreted cytologically as false negative since the individual cells are very apt to appear quite similar to normal cells. This is particularly true of some prostatic carcinomas and many carcinomas of the kidney. False negative cytologic evaluations also may occur when the specimens have not been handled properly; if specimens are left to stand for long periods at room tempera-

ture and without the addition of a preservative, the cellular components may degenerate beyond recognition by the time specimens are processed in the laboratory.

Fortunately, false positive reports are not often given by experienced cytologists. At times such reports are accounted for on the basis of marked cellular alterations produced by certain nonmalignant conditions, such as chronic cystitis or the presence of calculi. At other times, they may be simply the result of the cytologist's relaxation of his adherence to the criteria of malignancy. Conservatism in reporting a specimen as positive for cancer is recommended. With this policy it has been possible to restrict false positive cytologic interpretations to about 1 per cent.[7]

It should be kept in mind that the use of instrumentation for collecting specimens may cause displacement of cells from one site to a second one and that the examiner might make the mistake of assuming that the cells were derived from the second site. A case in point is one known instance where the tip of a ureteral catheter dislodged malignant cells from a bladder carcinoma near the ureterovesical orifice and carried them up to the renal pelvis. The malignant cells were then thought to have originated from the renal pelvis.

## Conclusions

The cytologic examination of genitourinary specimens for the purpose of detecting malignant neoplasms is a valuable addition to the more conventional diagnostic methods. It lends itself well to the discovery of early lesions and of those lesions which are beyond the reach of cystoendoscopy. The microscopic evaluation of cytologic preparations should be made only by individuals whose training qualifies them for this specialized work. The proper collection of specimens also carries great importance if the full value of the cytologic method is to be realized. For example, it has been shown that, in general, urine specimens are inadequate for the cytologic study of the prostate; prostatic secretions obtained by massage give much more accurate results in the determination of the presence of prostatic malignancy. The clinician should know the limitations of the method and collaborate closely with the cytologist in acquiring the most suitable material for study and providing pertinent information which can contribute to accurate diagnosis. Since some nonmalignant conditions may give rise to confusing cellular atypias, the presence of these disorders should be made known to the cytologist so that the issuing of false suspicious or false positive reports can be prevented.

It would probably be a mistake to assume that the currently used techniques for the collection and laboratory processing of cytologic specimens are the optimal procedures. Undoubtedly, further refinements will be developed which will enhance the usefulness of this diagnostic method and produce even higher degrees of accuracy in the detection of malignant neoplasms of the genitourinary tract.

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## Recommended Reading

ATLAS OF EXFOLIATIVE CYTOLOGY. By George N. Papanicolaou, M.D., Ph.D. Cambridge. Harvard University Press. 1954. SUPPLEMENT 2. 1960.

The recently published SUPPLEMENT 2 of this valuable *Atlas* includes color plates in two new series, the Vascular System and Other Body Fluids. Three new plates illustrate cells found in the circulating blood in eight cases of malignant neoplasms, mostly during or shortly after operations. A concise discussion of this new application of exfoliative cytology is given in Chapter X, which was written by Drs. Elizabeth A. McGrew, Stuart Roberts, Alvin L. Watne, Olga Jonasson and Warren H. Cole. The fourth new plate illustrates cytologic and histopathologic material from six cases in which malignant cells were recovered in spinal fluid.

The *Atlas* may profitably be used as a guide by students of cytology. Its main objective is to draw together into a single publication different forms of exfoliated cells—normal, atypical and abnormal—and have them described and illustrated under uniform standards that facilitate their comparative evaluation and help to appraise accurately their diagnostic potential.

# Keeping up

## Treatment of Cancer

Although there has been steady improvement in the cure rate of patients treated for almost any type of cancer, there has been no change in the death rate from cancer. The problem is to find better methods of treating the fatal cancers. Cancer cells exfoliate internally as well as externally and it is estimated that they are free in the blood of 40 to 78 per cent of patients with operable cancer. Probably all true cancers, at some time in their development and perhaps long before they become recognizable, have entered the blood stream and become disseminated. The fate of the individual patient may depend largely on whether or not metastases can implant and grow. Many factors influence the spread of cancer. It is not known whether lymph nodes are a source of further dissemination, whether they act as natural barriers, or whether they are important in the patient's immunological defense.

Animal experiments suggest that under certain circumstances operations may favor the growth of metastases, indicating that trauma may predispose to implantation of cancer. McWhirter has reported that when patients with breast cancer were subjected to radical and to simple mastectomies, and both groups were treated by X ray, a greater proportion of patients treated by the lesser operation survived. There is strong evidence that, in poorly selected cases, radical mastectomy may disseminate the disease. There is no scientific basis to justify advocating simple mastectomy, radical mastectomy, ultraradical mastectomy or irradiation as the single or even the chief method of treatment of breast cancer. Each of the different types of breast cancer deserves its own treatment. To distinguish between them is a challenge to the surgeon and needs more emphasis in cancer clinics.

*Crile, G., Jr.: Problems in the treatment of cancer. Maryland M. J. 7:467-471, Sept., 1958.*

## Hypophysectomy in Advanced Carcinoma

The effect of pituitary ablation in 64 patients with advanced malignant disease, 57 of whom had breast cancer, is reported. Surgical hypophysectomy was carried out in 52 patients; two had pituitary stalk section and three had radioactive seed implantation. The data presented in the text are summarized in 17 tables. Surgical hypophysectomy with the insertion of 15 mc of unscreened $Au^{198}$ or $Y^{90}$ seeds is considered the best method of pituitary ablation. Forty-two per cent of the total 52 patients who had surgical hypophysectomy, or 66 per cent of the 33 patients who survived three months or more showed objective improvement. Average length of remissions was 8.1 months, the longest being 27 months. There was a prolongation of life from an average of 3.2 months for patients showing no response to an average of 10.1 months for those showing improvement. Total ablation of all anterior pituitary tissue was rarely possible, but clinical improvement was observed with 10 to 20 per cent of tissue in the fossa. There was little evidence that regrowth of the pituitary

# with Cancer

stalk or of the gland parenchyma occurred. The results of postoperative tests of pituitary function are reviewed and correlated with postmortem examination of the pituitary fossa. The authors conclude that hypophysectomy is an effective method of palliating advanced metastatic cancer of the breast and prostate and that it is preferable to adrenalectomy but should not be considered a routine procedure. It may contribute materially to the elucidation of cancer metabolism and hormonal dependence, but the search for new forms of therapy must continue.

*Baron, D. N.; Gurling, K. L., and Smith, J. R.: The effect of hypophysectomy in advanced carcinoma of the breast. Brit. J. Surg. 45:593-606, May, 1958.*

### Physician Responsibility in Advanced Cervical Cancer

Despite commendable efforts to educate the profession and the public in the importance of early diagnosis and treatment of cervical cancer, management of patients with advanced cervical cancer will be a major problem until education is improved or more effective treatment is available for this stage of the disease. These patients deserve the same careful evaluation of the extent of disease as patients with early lesions. The following are essential: (1) biopsy of local or distant suspicious lesions; (2) chest X-ray study; (3) bone survey by X ray; (4) cystoscopic examination and pyelography; (5) proctoscopic examination and barium enema; (6) complete record of previous therapy. Patients should demonstrate failure to respond to irradiation before being considered candidates for pelvic exenteration. The latter should be considered only when studies indicate that it might be curative; it is questionable whether such extensive operation is justified for palliation. External irradiation is frequently helpful in controlling bleeding. Ligation of a single bleeding point in the vaginal tumor or of the hypogastric and ovarian arteries may be beneficial. Transfusions and supportive therapy are helpful in patients with widespread disease and profound anemia. A sigmoid or transverse colostomy may be done when there are rectal involvement and rectovaginal fistula. It is debatable whether any procedure for urinary diversion is beneficial (unless the tumor is resectable) when tumor has invaded the bladder causing vaginal drainage of urine. Management of ureteral obstruction is controversial. External irradiation may alleviate pain secondary to metastatic bone tumor and pelvic or leg pain due to involvement of the lumbosacral plexus. Cordotomy can relieve intractable pelvic and leg pain. If these methods fail, the physician must resort to adequate medication to make the patient comfortable.

*Thornton, W. N., Jr.: The responsibility of the physician to the patient with advanced cancer of the cervix. South. M. J. 52:306-308, March, 1959.*

### Changing Responsibility of Physicians

Complete and thorough examination of all patients who present themselves for medical attention is the basis of good medical practice. The physician must carry out the appropriate tests, if he is equipped to do so, or refer the patient to

someone who is. Periodic checkup should be performed for the purpose of unearthing clues to disease before it begins. The responsibility of the practicing physician today is changing from one strictly of treatment to one of health education and prevention of disease. It is important to make the diagnosis of cancer, but still more so to accomplish the diagnosis early while the disease is amenable to cure by complete eradication. Although the procedures of X-ray examination, proctosigmoidoscopy, exfoliative cytology and other methods of cancer detection and diagnosis are effective tools, it behooves research physicians and scientists to develop new approaches to determine cancer proneness or potential. We must think in terms of cancer prevention as well as cancer detection and treatment.

*Frohman, I. P.: The changing responsibility of the practicing physician in cancer detection. Postgrad. Med. 27:285-289, March, 1960.*

### Cancer of the Breast

To such slogans as early diagnosis and early treatment should be added another—that late cancer may also be curable. In the private practices of the authors from 1944 to 1955, 604 cases of cancer of the breast were seen, of which 284 were treated by radical mastectomy. Many of the other patients had recurrences and metastases after treatment elsewhere. Thirty-five, on admission, had primary cancers of the breast which were technically operable but were considered by the authors to be biologically inoperable. Of the patients treated previously, 48 had local recurrences, 151 had disseminated disease and 86 were seen only in consultation. Five-year survival of the patients with disease limited to the breast and treated by radical mastectomy was 82 per cent—higher than many other reports, by virtue of exclusion of biologically unfavorable cases, as evidenced by history of rapid growth and edema and erythema of the skin overlying the tumor. In Stage II surgical management may be actually harmful in cases that are biologically unfavorable as above and in addition have axillary nodes miliary in size but diffuse and extending to the apical area, or more than 2 cm in diameter, or fused to each other or to the skin. Five-year survival following standard radical mastectomy in the group of Stage II A cases, considered to be biologically favorable, was 55.8 per cent. Test for radiosensitivity is recommended for Stage II B cases — those with a greater extent or bulk of disease or with unfavorable signs of growth potential. Full courses of irradiation in those found to be radiosensitive yielded a five-year survival rate of 31.6 per cent. Radical mastectomy in the insensitive group yielded a rate of 19.4 per cent. The local recurrence rate in the whole group was only 5.3 per cent — due in part to filtering out the biologically unfavorable cases which were denied the very doubtful advantages of radical surgery. With complete exposure of the wound and constant suction drainage for the first two or three days, primary healing was obtained in 82 per cent of radical mastectomies. Minimum marginal necrosis with healing in 21 days or less occurred in 15 per cent, and major necrosis of skin flaps in only 3 per cent. Lymphedema of the arm is minimized by this method of wound management. Many of the 180 patients treated by irradiation were given supplemental hormone therapy. Surgical treatment of women more than 75 years of age has been discontinued.

The dominant importance of the inherent biologic characteristics of mammary carcinoma as a major factor in the results of treatment is that cancer which is historically and clinically late or advanced may be curable. It is best to fire the small therapeutic bullets first—hormones, oophorectomy, or pelvic irradiation, reserving the physiologic blockbuster of adrenalectomy for those patients who have demonstrated a real and objective response to earlier measures of treatment. These authors "can even conceive, with some difficulty, that the more hazardous insult to the organism, that of hypophysectomy, might even be of ultimate value in the responsive patient."

*Macdonald, I., and Guiss, L. W.: Carcinoma of the breast in a private practice. Arizona Med. 16:726-731, Nov., 1959.*

## Postoperative Irradiation of Cervical Cancer

This article attempts to determine the value of postoperative irradiation. The 86 cases reported comprise all the patients referred for postoperative X-ray therapy by the Gynecology Service (Memorial Center, New York) during the period 1948 through 1956. None of the patients had received preoperative irradiation. Case material was divided as follows: category I—the completely excised group (15 cases); category II—the postoperatively recurrent cancer group (27 cases); category III—the postoperatively persistent cancer group (24 cases)—this category was further subdivided into (A) proved persistent cancer (11 cases), and (B) presumed persistent cancer (13 cases); category IV—the explored group (20 cases). Better survival and palliation were evident in those categories in which there was no demonstrable residual disease at the completion of surgery. All patients undergoing exploration in whom cancer was not excised have died. The three categories of patients that have had better results yielding a few survivors alive and free of cancer at the time this report was prepared are as follows: four patients with no known residual postoperative cancer (category I); three patients with presumed persistent cancer (category III B); four patients with postoperative recurrent cancer (category II). Twenty-nine patients (34 per cent), irrespective of category, survived more than 18 months and experienced worthwhile palliation from irradiation. In the remainder of the patients the extent of the disease in the pelvis or the presence of para-aortic nodal metastases made radiation a last-resort measure. The study indicates that postoperative irradiation administered in the presence of proved disease can bring about five-year arrests and considerable palliation and prolongation of life to an otherwise incurable group of patients in whom further surgical efforts are of no value.

*Liegner, L. M.; Olson, M., and Nickson, J. J.: Postoperative irradiation of 86 cases of carcinoma of the cervix. Surg., Gynec. & Obst. 108:313-320, March, 1959.*

## Cytologic Detection of Uterine Cancer

Good preservation of representative cellular material is essential to uterine cytologic study. Two specimens are taken: the first is the cervical plug collected with a swab inserted into the canal, and the second is obtained with a wooden spatula, by rotating the smaller end in the canal. If the cervix is eroded, the specimen is collected from the border of the lesion with the broader end of the spatula. Microscopic findings are reported as follows: (1) tumor cells consistent with malignant tumor of the cervix or endometrium, (2) abnormal cells suggestive of malignant tumor, (3) atypical cells, probably associated with inflammatory lesion, (4) no abnormal cells. When tumor cells or cells suggestive of tumor are found, tissue is removed for histologic confirmation. If the lesion is visible, biopsy may be sufficient; if it is not, cervical conization is indicated. During the period 1955 through 1957, a total of 44,847 specimens were examined from 44,700 patients. Tumor cells consistent with malignant disease were found in 165 specimens. Forty-one specimens showed abnormal cells suggesting a malignant or atypical nonmalignant lesion. Examination of tissue from 131 of the 148 patients whose specimens showed tumor cells confirmed the diagnosis of malignant disease. Of the 41 patients in whom abnormal cells suggested malignant tumor, 14 had malignant lesions. Only one of 180 patients with atypical cells revealed a malignant lesion on histologic study. These findings indicate that in Nassau County (New York) the rate of detection of cervical cancer is four cases per 1000 of the female population for all ages. Fifty per cent of the malignant cervical tumors were carcinoma *in situ*. Sixty-five per cent of patients were between the ages of 20 and 40. Ten per cent of the confirmed malignant uterine lesions were endometrial. A significant observation was the lack of clinical findings in patients with intraepithelial cancer (carcinoma *in situ*).

*Maillard, E. R.: Cytologic detection of uterine cancer. New York J. Med. 59:1993-1997, May 15, 1959.*

## Fluorescent Stain in Cancer Detection

The acridine orange (AO) stain is specific for differentiation of RNA and DNA. The accumulation of RNA binds the fluorescent dye and produces a striking red or orange color in contrast to the green stain of the nuclear DNA. Malignant cells contain sufficient RNA in the cytoplasm to impart a bright red or orange fluorescence to the cells which makes them stand out prominently in stained smears. The classical morphological criteria of malignancy as demonstrated by the Papanicolaou technique has been well correlated with the interpretation of slides stained with AO.

In this study, the acridine orange differential nucleic acid stain was applied to benign and malignant, stock and fresh cell cultures, to determine whether this histochemical technique could differentiate benign from malignant origin. The biochemical phenomena of growth of both kinds of tissues were found to be too similar for the method to suffice as a single diagnostic technique. The AO stain is not specific for cancer cells.

*Kornfield, H. J., and Werder, A. A.: A differential nucleic acid fluorescent stain applied to cell culture systems. Cancer 13:458-461, May-June, 1960.*

## Detection of Genitourinary Cancer

In the treatment of genitourinary cancer, the cure rates achieved by radical surgery and irradiation have reached a plateau; improvement of cure rates must be found in earlier recognition and earlier treatment. The family physician is well equipped to perform periodic genitourinary examinations and if they are performed correctly, with proper emphasis placed on the findings obtained, his results can be identical to those of the urologic specialist. There are, however, certain aspects of examination which require more than routine attention. The most frequent finding in patients with kidney or bladder carcinoma is gross or microscopic hematuria which always warrants urologic investigation. Bladder carcinomas are often secondarily infected and may present signs or symptoms of recurrent cystitis. Cystoscopy and pyelography give reliable information and are more effective than the Papanicolaou smear. Since cryptorchid and atrophic testes are 50 times more prone to malignancy than normal testes, it would appear that all such unilateral testes in postpuberal males should be removed. Although the great majority of prostatic cancers begin in the area of the prostate which is palpable by rectal examination, patients with this disease are still not referred to the urologist early enough so that radical surgery can be attempted. Any nodule or area of increased density in the prostate of a man over the age of 45 should be considered to be cancer until proved otherwise.

*Baker, R.: Value of periodic examination in detecting cancer of the genitourinary system. Postgrad. Med. 27:301-305, March, 1960.*

The U. S. Public Health Service has recently awarded grants of $267,565 to six state and local health agencies to promote community cancer demonstration projects in their new program to further extend the use of present knowledge in preventing and controlling cancer.

The Advisory Committee of this Cancer Control Program feels that the following areas offer good opportunities for demonstrating better ways of providing community cancer control services: (1) professional and technical education in cytology; (2) screening of female patients for cancer of the cervix; (3) educational projects, such as public information and follow-up services to stress importance of periodic uterine cytologic examination; (4) professional education activities stressing importance of diagnostic aids in complete health examinations; (5) public education projects in desirability of and need for regular physical examinations; (6) evaluation of effectiveness of public education activities; (7) maintenance of tumor registries to collect data; (8) extension and evaluation of rehabilitation programs (in cooperation with state rehabilitation agencies); and (9) programs demonstrating effective treatment for cancer in beneficiaries of public medical care.

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Wayne State University College of Medicine and the Child Research Center of Michigan have established a cell bank for research in cancer, viruses and general biology. This bank is the third of its kind in the United States. It will operate under the direction of Dr. Cyril S. Stulberg, Associate Professor of Microbiology and Dr. Lawrence Berman, chairman of the Department of Pathology. Funds granted by the National Cancer Institute made possible the construction of the laboratory.

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A research team at the Albany Medical College (Albany, N. Y.) has been awarded an $80,000 grant by the National Cancer Institute to support, for the first three years, a study of the bronchial secretions of lung cancer patients. In their preliminary work, the group found that tumors of the lung could be produced in hamsters by injecting them with secretions from the lungs of cancer patients. The group will search for the responsible factor or factors present in the secretions of lung cancer patients.

At the Seventh National Medicinal Chemistry Symposium of the American Chemical Society, Dr. Allan L. Grossberg (Roswell Park Memorial Institute, Buffalo), reported that the active site of an antibody consists of amino acid residues arranged in such a manner as to form a configuration complementary to the shape of the antigen molecule and that the antibody "fits" the antigen.

Dr. David Pressman, also of Roswell Park, stated that another factor in antigen-antibody specificity is force. Although the forces may be weak individually they form a strong bond in combination, depending on the attraction of negatively and positively charged bodies. Drs. Grossberg and Pressman, both cancer researchers, are investigating the application of antigen-antibody chemistry to the destruction of neoplasms.

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The Veterans Administration is sponsoring a large number of research studies on the biologic aspects of aging of cells, organs and organisms in its program of investigating the medical problems of the aged. In the field of cancer research alone, there are over 250 projects currently in progress which have some relation to the aging problem.

In addition to these research activities, the VA is increasing the medical services for the aged and the chronically ill. It is setting up new geriatric clinics as well as day-care centers for the mentally-ill and is developing a community home care program for patients and a planned-living program for its domiciliary patients.

# COMING MEDICAL MEETINGS

| Date 1960 | Meeting | City |
|---|---|---|
| Aug. 2-4 | International Association of Laryngectomees | St. Louis |
| Aug. 7-12 | International Congress of Gerontology | San Francisco |
| Aug. 8-11 | National Medical Association | Pittsburgh |
| Aug. 11-13 | Rocky Mountain Radiological Society | Denver |
| Aug. 14-19 | International Congress of Clinical Chemistry | Edinburgh |
| Aug. 21-26 | International Congress of Physical Medicine | Washington, D. C. |
| Aug. 24-27 | International Congress of Internal Medicine | Basel |
| Aug. 27-Sept. 1 | American Hospital Association | San Francisco |
| Aug. 28-Sept. 1 | International Congress on Diseases of the Chest | Vienna |
| Aug. 28-Sept. 3 | International Congress of Histochemistry and Cytochemistry | Paris |
| Aug. 29-Sept. 3 | European Conference on Electron Microscopy | Delft |
| Sept. 3-10 | International Congress of Preventive Medicine and Social Hygiene | Vienna |
| Sept. 4-9 | International Society of Orthopedic Surgery and Traumatology | New York City |
| Sept. 4-10 | International Society of Hematology | Tokyo |
| Sept. 4-10 | World College of Anesthesiologists | Toronto |
| Sept. 5-9 | American Association of Blood Banks | San Francisco |
| Sept. 7-9 | Congress of International Society for Cell Biology | Paris |
| Sept. 7-9 | International Society of Geographical Pathology | London |
| Sept. 7-10 | Medical Women's International Association | Baden-Baden |
| Sept. 11-15 | International College of Surgeons | New York City |
| Sept. 13-15 | Fourth National Cancer Conference | Minneapolis |
| Sept. 15-22 | World Medical Association | West Berlin |
| Sept. 18-19 | New England Society of Anesthesiologists | Bretton Woods, N. H. |
| Sept. 22-26 | International Cancer Cytology Conference | Madrid |
| Sept. 23-25 | Inter-Society Cytology Council | Chicago |
| Sept. 24-27 | College of American Pathologists | Chicago |
| Sept. 24-Oct. 2 | American Society of Clinical Pathologists | Chicago |
| Sept. 26 | Society For Pediatric Radiology | Atlantic City |
| Sept. 27-30 | American Roentgen Ray Society | Atlantic City |
| Oct. 2-7 | American Society of Plastic and Reconstructive Surgery | Los Angeles |
| Oct. 2-7 | American Society of Anesthesiologists, Inc. | New York City |
| Oct. 6-8 | Central Association of Obstetricians and Gynecologists | Kansas City, Missouri |
| Oct. 10-13 | American Association of Medical Record Librarians | Seattle |
| Oct. 10-14 | American College of Surgeons, Clinical Congress | San Francisco |
| Oct. 17-22 | Symposium on Diagnosis and Treatment of Acute Radiation Sickness | Geneva |
| Oct. 23-26 | American College of Gastroenterology | Philadelphia |
| Oct. 23-29 | Pan American Congress of Gastroenterology | Santiago |
| Oct. 28-29 | Society of Pelvic Surgeons | Louisville |
| Oct. 30-Nov. 4 | American Public Health Association | San Francisco |



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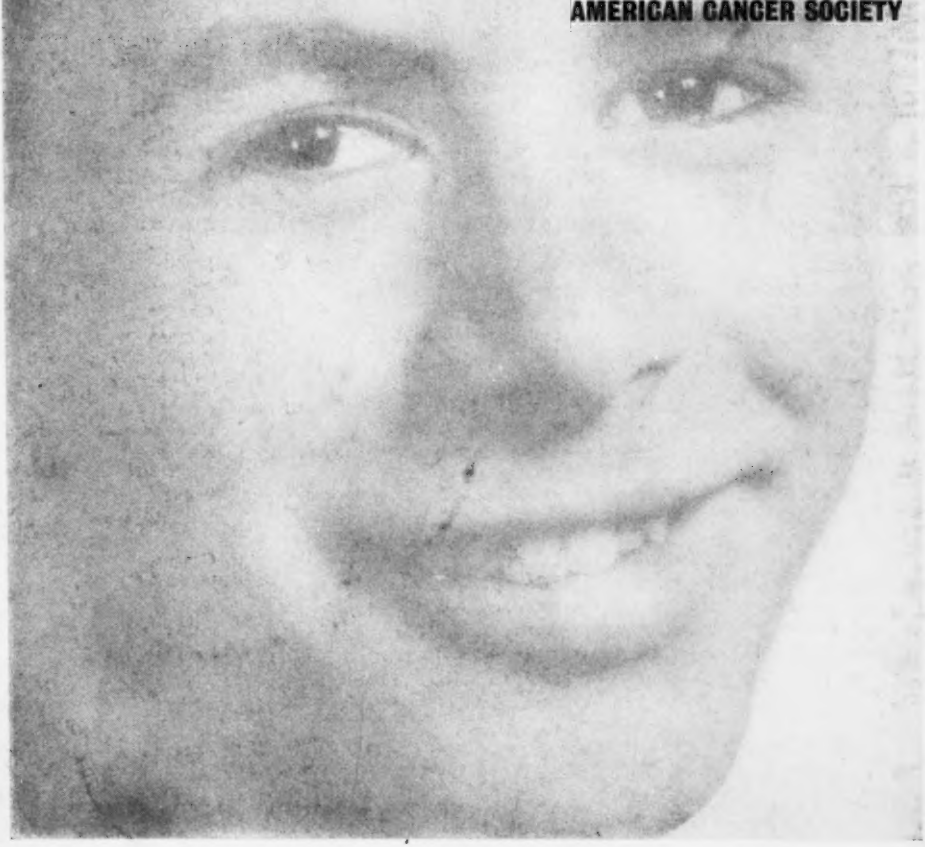
**MORE THAN A MILLION AMERICAN SCHOOL CHILDREN ARE DOOMED TO EVENTUAL DEATH FROM LUNG CANCER IF PRESENT TRENDS CONTINUE.** The American Public Health Association made this prediction last Fall and called upon "health authorities to undertake a broad educational effort, especially among young people, to prevent cigarette smoking."

How is this to be done, doctor? As a partial answer, the American Cancer Society has produced a filmstrip, TO SMOKE OR NOT TO SMOKE, based on a recent study of thousands of high school students. This filmstrip is available from the Society's 60 Divisions throughout the country.

The effectiveness of this educational tool will depend on the support of the entire community—teachers, parents and, most of all, physicians. Many physicians are participating in the presentation of the filmstrip.

This is another example of how the medical profession and the American Cancer Society can cooperate in essential public education in the fight against cancer.

**AMERICAN CANCER SOCIETY**